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rye to advance to the reproductive stage, and that low temperature of germination cannot be regarded as the initiating factor in flower production but is rather an accelerator.

The vernalization experiments with mustard to be described in this paper corroborate the conclusion that germination at low temperature acts as an accelerator. Vernalization technique depends on the discovery of the principle that as soon as the growth of the dormant embryo starts, the seed can be treated, from a physiological point of view, as a growing plant. In other words, similar development processes will occur whether the environmental requirements of temperature and light are pre-supplied to the germinated seeds and seedlings before they are sown or transplanted, or whether the plants obtain these under natural conditions in an advanced stage of their vegetative growth. In other words, by using pre-treated seeds of certain crops it is possible to raise these crops in a region or during a given season in which the necessary environmental factors are normally lacking. In discussing the possibilities of vernalized seeds for Indian agriculture, however, it has been shown [Sen, 1939] that except for transplanted crops the many theoretical possibilities of pre-supply of environmental factors are limited practically to the supply of low temperature.

Lysenko maintains that 'the processes conditioning the sexual reproduction of cereals may occur not only in growing plants, but also in a seed with an embryo which has just commenced development but not broken the seed-coat' [Imp. Bur. of Pl. Genetics, 1935]. So far as we are aware, however, all vernalization experiments described in the literature have been carried out with chilled seedlings—for germinated seeds during the period of chilling normally develop into seedlings—and not chilled seeds with intact seed-coats. In the case of Gramineae (wheat, oat, barley, etc.) seeds with emerged coleoptile and several roots can be dried and successfully re-germinated, but drying is fatal in the case of mustard seeds with emerged radicles. In our preliminary report on vernalization of mustard [Sen and Chakravarti, 1938], experiments have been described which show that, (a) plants from chilled seeds—like those which sprout during the period of chilling and those with intact seed-coat (unsplit seeds)—flower significantly earlier than plants from untreated control seeds, (b) for the same dose of chilling, the earliness in flowering is greater in plants from sprouted vernalized seeds, and (c) despite greater earliness that can be obtained from sprouted vernalized seeds, only unsplit vernalized seeds of mustard offer practical agricultural possibilities, since the sprouted chilled seeds have to be sown with great care for the reason that drying is fatal for them, while, on the other hand, unsplit seeds can be dried and stored without any loss of subsequent germinating capacity.

The results of the past four years' vernalization experiments with mustard at Almora (United Provinces) are described in the present paper. Most of the experiments were carried out with mustard Type 27 from New Delhi, but vernalization responses of mustard Types 9 and 11 from Cawnpore, and of yellow *sarson* and *raya* O.B/I from Lyallpur, have also been observed.

Working with winter wheat, Lojkin [1936] found that drying induced devernalization, and Gregory and Purvis [1938] observed similar reversal of vernalization induced by drying of vernalized grains of winter rye. Our

first problem, therefore, after our preliminary experiments [Sen and Chakraborti, 1938], was to find out whether vernalized unsplit seeds of mustard, when dried and stored for a minimum period necessary for the practical requirements of distribution and sowing, would retain unimpaired the effect of chilling. After the encouraging result of the first experiment of 1938, which showed that drying of unsplit chilled seeds up to 9 days—a likely minimum period required for distribution and sowing—did not impair the induced vernalization, experiments were undertaken to find out: (1) The optimum conditions and period of chilling necessary to induce maximum vernalization in unsplit chilled seeds of mustard. (2) Vernalization response of different strains of mustard. (3) Effect of vernalization on the progeny. (4) Period or which unsplit chilled seeds could be dried and stored without any loss of induced vernalization. (5) Effect of after-sowing temperature and day-length on the vegetative period of plants from control and vernalized seeds.

MATERIAL AND METHOD

The technique of vernalization previously described by Sen and Chakraborti [1938] has been found to be very satisfactory for small samples of seeds. For vernalizing larger samples necessary modifications were introduced, particularly in regard to the containers of seeds and provision for absorption of CO_2 from the respiring seeds. The seeds to be chilled are first soaked under excess of water to make them absorb about 60 per cent of their weight of water, which generally takes six to eight hours, according to the room temperature. After removal of excess water by spreading the seeds over several layers of absorbent cloth, they are put in muslin bags or unglazed porcelain pots of suitable sizes and are then placed inside the moist-chamber of the chilling-cabinet.

Any watertight box of required dimensions with removable lid can be used for a moist-chamber. When boxes of thin wood are used, they should be thoroughly asphalted inside and out. The inside of the box is lined with blotting paper and sufficient water is placed at the bottom of the box to maintain the absorbent lining moist throughout the period of chilling. For absorption of CO_2 , a concentrated solution of KOH is kept at the bottom of the box in a large, flat porcelain dish, the rim of which is previously paraffined to prevent creeping of KOH solution. A removable thick wire-net frame is placed over the KOH dish to protect the seeds against any accidental contact with the solution. Seeds in bags are suspended from hooks screwed on to the removable lid of the box, care being taken to see that the suspended bags do not touch the wire-net guard, or the moist blotting-paper lining of the box. When unglazed porcelain pots are used as seed containers, they are placed on the wire-net guard above the KOH solution. From daily readings of the maximum-minimum thermometer, the temperature range to which the seeds are subjected is recorded. Obviously, from these readings no definite idea is obtainable about the duration of the recorded temperatures each day.

Chilling-cabinet

An electrically operated cabinet of the Frigidaire type with an automatic device for maintaining a constant low temperature is undoubtedly the most

suitable appliance for chilling seeds. Since Almora has no electric supply, we used a kerosene-operated Electrolux for our experiments. An ordinary ice-box can, however, be used for chilling seeds, and when the low temperature required is not below 5°C. and only small samples of seeds are to be chilled, even a wide-mouthed thermos-flask can be used very successfully for chilling seeds in the following way. The thermos-flask is half-filled with freezing mixture and the soaked seeds are hung in a muslin bag from a hook screwed on the underside of the cork stopper of the flask. The process of daily renewal of the freezing mixture insures the necessary removal of CO₂ and a supply of fresh air. Additional moisture when required can be given to the seeds by dipping the bags in ice-cold water—the excess water automatically drips down into the flask. As will be seen later, mustard seeds thus chilled in a thermos-flask gave excellent results.

After the required periods of chilling, the sprouted mustard seeds are discarded and the unsplit seeds are washed and dried at room temperature till they attain a constant weight. The period varies from three to five days, according to the season. The seeds are then packed in a sealed container and stored inside the Electrolux.

Sowings were done both in pots filled with thoroughly sifted garden soil and in well-prepared field plots. When there were only two variables, i.e. one treatment each of vernalized and control seeds, they were sown in two halves of the same pot. Four plants (two of each) were grown in one pot (9 in. diameter). For preliminary trials two or three pots were used for each sowing. In final trials four pots for each variable were used. For field-plot trials four to six replications were used. To avoid errors of observation and possible injury to the growing point involved in determining the date of emergence of the first flower-buds, the date of the opening of the first flower—a strikingly visible phenomenon—was arbitrarily taken as the end of the vegetative period. The observed percentage of shortening of the vegetative periods (from sowing to opening of first flower) of plants from chilled seeds compared to those of control plants was taken as the measure of the vernalization response.

EXPERIMENTS

EXPERIMENT 1

As has already been stated, the first experiment carried out in 1938 was to determine whether chilled unsplit seeds of mustard when dried for a period of about a week would at all retain the induced vernalization. This experiment being of a preliminary nature, seeds from a batch chilled for 30 days were sown: (i) immediately after removal from chilling cabinet, on May 26, 1938; (ii) after drying for three days, on May 29; (iii) after drying for seven days, on June 2; and (iv) on June 4, after drying for nine days. The first three sowings were in pots and the fourth, replicated four times, was in well-prepared small garden plots. The control seeds used for the first sowing were previously soaked under water for six hours, and for the rest of the sowings, ordinary dried seeds were used. Control and vernalized seeds were sown in the two halves of each pot.

It will be seen from the data summarized in Table I that in all the four sowings, plants from unsplit chilled seeds flowered significantly earlier than

those from control seeds grown under similar after-sowing conditions. The vegetative periods of plants from V-seeds in all the three pot sowings were similar. The greater percentage of shortening observed in the first sowing (fresh chilled seeds) was due to the increased vegetative period of the plants from the C-seeds. From this experiment the conclusion is justified that unsplit chilled seeds of mustard when dried up to nine days—the likely minimum period required for distribution of seeds—will produce plants which will flower earlier than the untreated control.

TABLE I

Vegetative period of mustard plants : C, from control seeds, and V, from vernalized unsplit seeds

(Period of chilling, 30 days)

Sowing date (1938)	Average vegetative period (days)*	Period of drying of V-seeds (days)	Shortening of vegetative period of V-plants (per cent)
3/5 . .	C— 47.0 ± 2.05 (3) V— 35.0 ± 0.75 (5)	0	25.5
9/5 . .	C— 41.8 ± 0.69 (10) V— 36.0 ± 0.63 (16)	3	13.9
/6 . .	C— 42.6 ± 1.06 (13) V— 35.0 ± 1.2 (12)	7	17.8
/6** . .	C— 53.6 ± 1.39 (37) V— 38.7 ± 0.73 (40)	9	27.7

* In this and in subsequent tables, figures within parantheses indicate the number of plants the mean of which is given.

** Sown in small field plots.

EXPERIMENT 2

To determine the period of chilling required for inducing maximum vernalization in unsplit chilled seeds, different batches of seeds were placed in the chilling-cabinet on appropriate dates to obtain, on July 1, 1938, batches of seeds chilled for eight, six, four and three weeks, respectively. These were all dried for 10 days and were sown, along with the controls, in four pots each on July 11, 1938. On this date batches of seeds chilled for four weeks but dried for 23 and 45 days were also available, and they were also sown in two pots each, to find out the effect of prolonged drying of unsplit chilled seeds.

The observed results are summarized in Table II. It will be seen that in all treatments plants from V-seeds flowered significantly earlier than those from C-seeds. The maximum earliness observed was from seeds chilled for six weeks (Plate I, fig. 2). The difference observed between treatments of eight, four and three weeks' chilling is not statistically significant, but the vegetative period of plants chilled for four weeks differs significantly only

from those of six weeks' chilling and not from eight and three weeks' chilling. Therefore, it was tentatively assumed that chilling for six weeks would induce maximum vernalization in unsplit seeds. It will also be seen that there is no significant difference in the vegetative periods of plants from seeds chilled for four weeks but dried for 10, 23 and 45 days. The fact that the seeds chilled for six weeks showed a higher degree of vernalization compared to those chilled for eight weeks clearly indicated that either, (i) the conditions of chilling were the optimum in the batch chilled for six weeks, or (ii) chilling beyond six weeks had induced devernialization. Therefore in 1939 investigations were undertaken to find out the optimum chilling conditions and also the effect of prolonged chilling.

TABLE II

Effects of different periods of chilling and drying

(Sowing date, July 11, 1938)

Period of chilling (weeks)	Period of drying (days)	Vegetative period (days)	Shortening of vegetative period in V-plants (per cent)
Nil (control) .	..	44.9 ± 1.17 (15)	..
3 . . .	10	37.0 ± 0.73 (15)	17.6
4 . . .	0	41.3 ± 1.5 (7)	8.0
4 . . .	10	37.8 ± 0.85 (16)	15.8
4 . . .	23	38.0 ± 1.17 (8)	15.3
4 . . .	45	39.5 ± 0.81 (8)	12.0
6 . . .	10	34.7 ± 0.96 (16)	22.7
8 . . .	10	37.0 ± 0.97 (15)	17.6

Analysis of variance

Due to	D. F.	Sum of sq.	Mean sq.	Ratio observed
Between treatment .	7	984.57	140.65	
Within treatment . .	92	1286.00	13.98	10.05 **
Total .	99	2270.57

S. E. per plant 3.74

**Significant at 1 per cent level

Optimum chilling conditions

In the case of winter wheat, it has been shown by Lojkin [1936] that the degree of vernalization induced increases with a greater rate of life activity within the seeds during the process of chilling. Sen and Chakravarti [1938] have also found this to be equally true in the case of mustard. Working with excised embryo of winter rye, Gregory and Purvis [1938] have clearly demonstrated that the reactions involved in the process of vernalization are localized in the embryo of the seeds. Obviously, the rate of life activity of the embryos of seeds which sprout during the process of chilling must be greater than that of seeds which remain unsplit. The fact that when a batch of soaked seeds of mustard is chilled varying proportions of both sprouted and unsplit seeds are obtained, indicates that, (i) all the seeds of a given sample are not similar in regard to their speed of germination, or (ii) that during the process of chilling all the seeds are not subjected to identical environmental factors of temperature, moisture supply and oxygen tension, or (iii) a combination of both (i) and (ii). That the speed of germination of individual seeds of a given sample of mustard varies, is seen from the fact that even at room temperature (20°-25°C.) when soaked seeds are spread over moist blotting paper in covered petri dishes in a single layer in batches of 50, it generally takes 14-15 hours from the sprouting of the first seeds in each batch until all the seeds in the batch have sprouted. At lower temperature (5°-10°C.) this period is increased on an average to 15 days. Since we have been able to recover unsplit mustard seeds from samples chilled for 365 days, it is evident that germination speed of individual seeds is not similar, and seeds densely piled in bags for chilling are obviously not subjected to uniform environmental factors. No attempt has been made to overcome this problem, since it is due to this very lack of uniformity, alike of the speed of germination and of the environmental conditions, that we have been able to recover unsplit chilled seeds. Furthermore, the seeds which sprout during the process of chilling offer the only visible indication that life activity is being maintained in the samples as a whole. Experiments have been undertaken, however, to find out the optimum temperature range and moisture supply for obtaining maximally vernalized unsplit seeds.

EXPERIMENT 3

For lack of an automatic device for maintaining different low temperature ranges, advantage was taken of the definite temperature gradient which exists inside a kerosene-operated Electrolux. Three different batches of mustard seeds (2.5 gm. each), after being soaked under water for six hours, were kept on the three shelves of the Electrolux, in three moist-chambers within which high humidity was maintained. From the daily record of the three maximum-minimum thermometers kept on the three shelves, the average temperature for the period of chilling (31 days) was obtained. The chilled unsplit seeds were dried at room temperature till they attained a constant weight, and each sample was weighed to determine the percentage of recovery of unsplit seeds. The results of sowings of these seeds on September 11, 1939, are given in Table III. The statistical analysis of the data shows that compared to the plants from the three samples of unsplit vernalized seeds,

chilled at different temperature ranges, the plants from untreated control seeds flowered significantly later, and that the differences observed in the vegetative periods of plants from different batches of vernalized unsplit seeds were not significant. Thus it would appear that a temperature range between 2° and 12°C. can be successfully used for chilling mustard seeds, but the higher the temperature the smaller will be the recovery of unsplit chilled seeds.

TABLE III

Effect of chilling at different temperature ranges with similar moisture supply, and percentage of recovery of unsplit chilled seeds

(Sowing date, September 11, 1939)

Mean temperature (°C.)	Vegetative period (days)	Recovery of un- split seeds (per cent)	Shortening of vegetative period of V-plants
Control	64.4 ± 2.39 (10)
Top shelf 3.5—12 .	45.6 ± 3.10 (10)	7.2	18.8 days, or 29.2 per cent
Middle shelf 2—10 .	45.4 ± 2.42 (9)	14.4	19.0 days, or 29.5 per cent
Bottom shelf 0—6 .	47.3 ± 2.73 (8)	32.0	17.1 days, or 26.5 per cent

Analysis of variance

Due to	D. F.	Sum of sq.	Mean sq.	Ratio observed
Between treatment .	3	2576.47	858.82	11.49 **
Within treatment .	33	2464.80	74.69	
Total .	36	5041.27

S. E. per plant 8.64

** Significant at 1 per cent level

EXPERIMENT 4

Under otherwise similar conditions, the rate of life activity of the embryo of mustard seeds increases, within limits, with increased supply of moisture, and therefore for the same dose of chilling the vernalization induced will vary according to the moisture supply. But to obtain vernalized unsplit seeds the embryo must not be allowed to grow beyond the elastic limit of the seed-coat. Though it has not yet been possible for us to determine the critical stage of

rowth of the embryo at which the seed-coat will burst, some rough estimate as been obtained about the effect of high and low humidity supply on induced vernalization under similar low temperature range. The variation of moisture supply was obtained by using different seed containers. For his experiment batches of 250 gm. of seeds were used for each treatment. For low humidity, two batches of previously soaked seeds were placed in unglazed porcelain pots and no additional water was given during the period of hilling; for high humidity, five batches of seeds were put in muslin bags and every week the bags were dipped in ice-cold water. Both sets of seeds were kept in the same moist-chamber. The seeds in unglazed porcelain pots were placed on top of the wire-net guard of the moist-chamber, and the muslin bags were hung from the hooks attached to the removable lid of the moist-chamber. The two batches of seeds in unglazed porcelain pots were chilled for longer periods than any used for batches in muslin bags. The various batches were placed in the chilling-cabinet at appropriate dates so that by September 22, 1939, the samples subjected to low humidity had been chilled for 14 and 12 weeks, and those subjected to high humidity for ten, eight, six, four and two weeks. After removing the sprouted seeds, the unsplit chilled seeds of all the batches were dried at room temperature for 10 days and weighed. These seven samples of chilled unsplit seeds were sown along with the untreated control seeds in well-prepared field plots. Four replications were used for each of the variables. The vernalization response of the different samples of chilled seeds and the percentage of recovery of the unsplit seeds from each sample are given in Table IV, and further details of this sowing are given later (Table IX) along with the statistical analysis of the data.

TABLE IV

Vernalization response of unsplit seeds chilled at low and high humidity and the recovery percentage of unsplit seeds
(Sowing date, October 2, 1939)

Period of chilling (weeks)	Humidity	Vernalization response (per cent)	Recovery of unsplit seeds (per cent)
2	High	10.05	78.0
4	"	14.24	58.2
6	"	30.26	33.5
8	"	28.46	40.0
10	"	31.37	34.0
12	Low	13.86	60.0
14	"	25.16	67.0

The observed differences in vernalization response of samples chilled for six, eight and ten weeks with high humidity and 14 weeks with low humidity are not statistically significant. This shows that chilling for six weeks under optimum conditions induces maximum vernalization in unsplit seeds and that chilling under similar conditions for 10 weeks does not induce any devernalization. Though chilling with high moisture supply for only six weeks induces maximum vernalization comparable to that induced by chilling for 14 weeks with low moisture supply, the percentage of recovery of unsplit seeds in the case of low humidity treatment is nearly double the figure for high humidity treatment.

EXPERIMENT 5

The conclusions of experiments 3 and 4 explain why the unsplit seeds chilled in a thermos-flask were maximally vernalized. An experiment was undertaken earlier to explore the possibility of utilizing simple devices for vernalizing mustard seeds. A temperature range of 4° — 8°C . and maximum humidity can be obtained for chilling seeds inside a wide-mouthed thermos-flask (such as is used commonly as a food-jar) half filled with freezing mixture. A batch of mustard seeds previously soaked under water for six hours was chilled in a thermos-flask for 52 days. The freezing mixture was renewed daily, thus assuring an adequate supply of oxygen for the seeds. The unsplit seeds chilled in a thermos-flask, after being dried for three days, were sown in pots on April 24, 1939, along with maximally vernalized unsplit seeds (chilled for 167 days) and the untreated control. The vegetative period of plants from control seeds was 41.5 ± 1.95 days (mean of four plants), while the periods of plants from seeds chilled in the thermos-flask for 52 days and chilled in the Electrolux for 167 days were found to be similar, being 31.0 ± 0.79 days (mean of five plants) and 31.9 ± 0.54 (mean of eight plants), respectively.

Another preliminary experiment was undertaken to find out whether the ground temperature of Almora (2° — 8°C .) could be used during winter for chilling seeds as a cheap method of vernalization. Chilling for 35 days under frost-covered ground produced a significant vernalization response, but the results showed that the seeds were not completely vernalized, as the shortening of the vegetative period from maximally vernalized seeds, sown on the same date, was 25.6 per cent, while those of plants from seeds chilled by the ground temperature for 35 days was only 12.7 per cent. From this experiment it is evident that at least partial vernalization can be obtained without the cost of operating a chilling-cabinet. The possibility of obtaining maximally vernalized unsplit mustard seeds is being explored.

EXPERIMENT 6

In the case of winter wheat it is reported [Imp. Bur. of Pl. Genetics, 1935] that seeds can be vernalized by instalment. For instance, instead of chilling continuously for 50 days, seeds may be chilled for 40 days, kept in a dry state until required, and then given a further period of chilling for 10 days before sowing. On the other hand, Gregory and Purvis [1938] have shown that 'as far as tendency to flower is concerned, the vernalized seeds of rye dried for 20 weeks are identical with unvernallized control.' In

he case of mustard, it has already been shown (experiment 2) that drying of unsplit chilled seeds up to 45 days does not affect the induced vernalization. The following experiment was undertaken to find out : (i) whether drying of partially vernalized unsplit mustard seeds for a period of more than 20 weeks would induce complete devernalization ; (ii) whether partially vernalized unsplit mustard seeds could be re-chilled to induce maximum vernalization ; and (iii) whether prolonged chilling would induce devernalization.

Plants from a batch of seeds chilled for six weeks from August 23 to October 4, 1939, dried for 10 days and sown in a small field plot showed a significant shortening of the vegetative period of only 7.2 per cent, a percentage considerably lower than could be expected from maximally vernalized unsplit seeds. This batch of seeds was stored in the Electrolux in a sealed bottle. On December 18, 1938, a sample from this batch of stored chilled seeds was soaked under water for six hours and re-chilled in the usual way till March 4, 1939. Still another batch of seeds was chilled uninterruptedly from October 25, 1938 to March 4, 1939. Both the re-chilled and continuously chilled unsplit seeds were dried for seven days. Thus, on March 11, 1939, the four batches of seeds sown were : (1) chilled for six weeks, dried for 158 days ; (2) chilled first for six weeks and then, after drying for 74 days, re-chilled for a further period of 77 days (the combined period of chilling being 119 days) and re-dried for seven days ; (3) chilled continuously for 129 days and dried for seven days ; and (4) control. From the observed data given in Table V it will be seen that : (i) incompletely vernalized unsplit seeds of mustard when dried and stored for 158 days can retain the effect of chilling, for they produced plants which flowered significantly earlier than the control plants ; (ii) incompletely vernalized unsplit seeds can be re-chilled after a period of drying for 74 days, to induce maximum vernalization, for the difference observed between Nos. 1 and 2 is statistically significant ; (iii) continuous chilling for a period of 129 days did not produce any injurious effect on the unsplit seeds, at least as far as the vernalizing reactions were concerned.

TABLE V

Effects of re-chilling incompletely vernalized unsplit seeds and of continuous prolonged chilling on induced vernalization

(Sowing date, March 11, 1939)

Nos.	Treatment of seeds	Vegetative period	Shortening of vegetative period in V-plants (per cent)
1	Chilled 6 weeks, dried 158 days .	41.77 ± 0.46 (13)	4.5
2	Chilled 6 weeks, after drying 74 days re-chilled 77 days and then dried 7 days	38.14 ± 0.25 (14)	12.8
3	Continuously chilled 129 days .	38.17 ± 0.56 (12)	12.7
4	Control	43.75 ± 0.56 (12)	..

These results indicate that it is possible to utilize any natural winter ground temperature ranging from 1° to 12°C. for vernalization of mustard seeds, since even if the cold temperature available in any given region be for a period not long enough to induce maximum vernalization, the partially vernalized unsplit seeds can be dried and stored for subsequent re-chilling by artificial low temperature at a convenient date.

Vernalization response of different strains of mustard

Preliminary experiments were undertaken to find out whether strains of mustard other than Type 27 would also respond to vernalization. Two strains of mustard from Cawnpore, Types 9 and 11, two strains from Lyallpur, *raya* O.B/I, and yellow *sarson*, were tried. It was found that unsplit chilled seeds of all these strains of mustard produced plants which flowered earlier than the plants from untreated control seeds. Compared to C 11, the vernalization response of C 9 was considerably greater. In the Lyallpur strains, in a sowing of September 6, 1938, the percentage of earliness observed in the opening of the first flower of plants from unsplit seeds of *raya* O.B/I and of yellow *sarson*, both chilled for 30 days and dried for 10 days, was 19.8 and 39.7, respectively.

EXPERIMENT 7

A sowing was undertaken to find out the comparative vernalization responses of mustard Type 27, C 11 and C 9. Samples of these strains were chilled for 52 days, and after drying at room temperature for four days the unsplit chilled seeds were sown, along with their respective controls, on August 11, 1939. From the results summarized in Table VI, it will be seen that with similar pre-chilling treatment of seeds and under similar after-sowing environmental conditions the percentage of shortening was the greatest in plants from chilled unsplit seeds of Type C 9 and lowest in Type C 11.

TABLE VI

Comparative vernalization responses of mustard Type 27, C 9 and C 11

(All chilled for 52 days, dried for 4 days and sown on August 11, 1939)

Strain	Vegetative period (days)	Shortening of vegetative period in V-plants
C 11	C—40.16±0.64 (12) V—31.82±1.3 (11)	8.34 days or 20.76 per cent
Type 27	C—59.9±2.06 (10) V—37.55±0.92 (9)	22.35 days or 37.31 per cent
C 9	C—61.44±2.93 (9) V—30.18±1.53 (11)	31.26 days or 50.88 per cent

Effect of vernalization on the progeny

EXPERIMENT 8

To find out whether the effect of vernalization is transmitted to the progeny, four different sowings were undertaken, and the vegetative periods of plants from seeds collected for three successive generations of control and vernalized seeds were observed. In these observations it was assumed that if the effect induced by chilling of seeds is transmitted to the progeny, then the plants from seeds collected from the very first generation of vernalized seeds would produce at least partially vernalized seeds. If, however, the effect transmitted to the progeny be of an undetectable intensity in the first generation, then vernalization of the progeny of vernalized seeds for three successive generations might be expected to give some visible indications. The first sowing was undertaken to collect the progenies of control and vernalized seeds. In the second sowing, the progenies of the control and vernalized seeds were vernalized and sown along with their respective untreated controls. This process was continued for the third and fourth sowings. The observed vegetative periods in all these sowings are given in Table VII.

TABLE VII

Vegetative periods of plants from three successive generations of vernalized and control seeds

No.	Seed stock	Sowing date	Period of chilling (days)	Mean vegetative period (days)	Significance
1	Original stock	June 4, 1938	..	C—53.6±1.39 (37)	
			30	V—38.7±0.75 (40)	**
2	Progeny of 1	May 15, 1939	..	cC—41.7±1.50 (12).	
			..	vC—41.0±0.52 (12)	Not sig.
			50	cV—28.4±0.64 (11).	
			50	vV—30.3±0.51 (12)	Do.
3	Progeny of 2	Sept. 2, 1939	..	ccC—71.3±2.53 (7).	
			..	vvC—74.7±3.45 (4)	Do.
			30	ccV—34.8±2.21 (5).	
			30	vvV—35.2±1.72 (5)	Do.
4	Progeny of 3	June 5, 1940	..	cccC—40.4±0.9 (8).	
			..	vvvC—47.1±0.7 (8)	**
			51	cccV—33.8±1.39 (7).	
			51	vvvV—39.6±1.62 (8)	**

** Significant at 1 per cent level

For abbreviation, the generations of the seeds used are indicated by small letters (c, control, and v, vernalized). The particular treatment given in each sowing, i.e. control or vernalized, is indicated by capital letters (C, untreated control, and V, vernalized). Thus, for example, control seeds used

for the third sowing are indicated as ccC and vvC. They are untreated progenies of the second generation of control and vernalized seeds. Similarly in the fourth sowing, vernalized seeds of the third generations are indicated as cccV and vvvV.

Except in the third sowing only, unsplit vernalized seeds were used for the vernalization test. Sprouted vernalized seeds had to be used for the third sowing because not only was the quantity of seeds we could collect from the second sowing very small, but the quality of the seeds appeared to be poor as well, and we could not be sure that unsplit chilled seeds of this sample would be viable. Both the sprouted control and vernalized seeds of the third sowing produced normal seeds, however, which were used for the fourth sowing, when again unsplit chilled seeds were used. From Table VII it will be seen that in sowings 2 and 3 progenies of control and vernalized seeds, alike untreated and vernalized, produced plants with similar vegetative periods, since the observed differences are not statistically significant. In sowing 4, however, the differences observed in the vegetative periods of cccC- and vvvC-plants and cccV- and vvvV-plants are statistically significant, but it was the ccc-seeds which produced plants which flowered earlier than those from vvv-seeds. Thus, it can be concluded that the effect of vernalization, as far as earliness in flowering is concerned, is not transmitted in the case of mustard Type 27 up to the third generation. The significant earliness observed in flowering of plants from ccc-seeds was due not to any cumulative inheritance nor to deterioration of vvv-seeds but, as will be seen from the following preliminary experiments, to the lower temperature range during the period of development and maturity of the seeds of ccC-plants, the first flowers of which opened from November 2 to November 24, while the corresponding period of the vvV-plants was from October 2 to October 14.

EXPERIMENT 9

It has been observed by Kostjucenko and Zarubalio [1937] that wheat seeds which develop and mature under low temperature become naturally vernalized. Gregory and Purvis [1938] actually produced vernalized winter rye seeds by chilling the ear. Mustard is a winter crop. In comparison with the Delhi region, Almora has a much colder winter, and as has already been shown the winter ground temperature of Almora can in fact be utilized for vernalization of mustard (Experiment 5). If low temperature during seed reproduction can induce at least partial vernalization, then, (i) untreated mustard seeds of the normal Almora harvest would be expected to produce plants which would flower earlier than those from the normal Delhi harvest, and (ii) Almora summer temperature being considerably higher than that of the Delhi winter, seeds reproduced in Almora from off-season sowings would be expected to produce plants which would flower later than plants from the normal Delhi harvest. The results of different sowings given in Table VIII indicate that low temperature during seed ripening will produce partially vernalized seeds; for in sowings Nos. 1, 2 and 3 the seeds from normal Almora harvest produced plants which flowered significantly earlier than those from the Delhi normal harvest, but in sowing 4, Almora summer seeds produced plants which flowered later than those from the normal Delhi harvest.

TABLE VIII

Vegetative periods of plants from seeds reproduced under different temperature ranges in Delhi and Almora

Nos.	Date of sowing	Seeds	Vegetative period (days)	Earliness (days)	Significance
1	Sept. 26, 1940	Normal harvest	Delhi — 60.77 ± 1.4 (13) Almora— 55.1 ± 1.46 (11)	5.67	**
2	Feb. 25, 1941	„	Delhi — 46.58 ± 0.36 (12) Almora— 44.72 ± 0.61 (11)	1.86	*
3	June 14, 1941	„	Delhi — 53.71 ± 1.67 (14) Almora— 48.75 ± 1.41 (12)	4.96	*
4	Sept. 11, 1940	Summer harvest Normal harvest	Almora— 62.7 ± 1.4 (12) Delhi — 53.2 ± 0.58 (9)	9.5	**

* Significant at 5 per cent level ; ** Significant at 1 per cent level

Vegetative period of plants from vernalized seeds under field conditions

The observations so far described were carried out mostly in pot cultures. Author series of experiments was undertaken to find out the shortening of the vegetative period that can be obtained by the use of vernalized mustard seeds grown under field conditions. Three strains of mustard—Type 27, C 11 and C 9—were used. The effect of different periods of chilling and also the effect of different periods of drying of unsplit vernalized mustard seeds were observed. With the co-operation of Dr T. S. Sabnis and of Dr B. P. Pal vernalized unsplit seeds sent from Almora by post were given field-plot trials in Cawnpore and New Delhi respectively. In Cawnpore all the three strains were tried and in New Delhi only Type 27 was used.

EXPERIMENT 10

The effect of different periods of chilling on the degree of vernalization induced was observed by simultaneous sowings of different batches of seeds chilled for different periods. Seeds of mustard Type 27, C 11 and C 9 were placed in the chilling-cabinet on appropriate dates so that on September 23, 1939, various batches of Type 27 seeds were obtained which had been chilled for 14, 12, 10, 8, 6, 4 and 2 weeks, respectively, and batches of C 11 and C 9 seeds, which had been chilled for 14, 10, 6 and 2 weeks. All unsplit chilled seeds of all batches of each strain were dried for the same period before they were sown along with their untreated controls in well-prepared field plots. Four replications were used for each treatment. The results obtained are given in Tables IX, X, and XI. It will be seen from the statistical analysis of the data that in Type 27 and in C 11 chilling for six weeks induces maximum vernalization in unsplit seeds, and further increase in the period of

chilling does not induce any higher degree of vernalization, neither any devernalization (Tables IX and X). In the case of C 9, chilling for a period longer than six weeks is necessary to induce maximum vernalization in unsplit seeds, since the vegetative period observed in plants chilled for 10 weeks is significantly shorter than in those from seeds chilled for six weeks (Table XI). The increased vegetative period observed (Table IX) in plants from seeds chilled for 12 weeks compared to those from seeds chilled for 6, 8, 10 and 14 weeks is due, as has already been explained (Experiment 4), to the reduced moisture supply.

TABLE IX

Effect of different periods of chilling (mustard Type 27)

(Unsplit chilled seeds dried for 9 days, sown October 2, 1939)

Period of chilling (weeks)	Vegetative period (days)	Earliness.	
		Days	Percentage
Control	80.96 ± 1.27 (47)
2	72.82 ± 1.38 (50)	8.14	10.1
4	69.43 ± 1.73 (46)	11.53	14.2
6	56.46 ± 1.06 (45)	24.5	30.3
8	57.92 ± 0.75 (48)	23.04	28.5
10	55.56 ± 0.88 (46)	25.4	31.4
12	69.74 ± 1.0 (47)	11.22	13.9
14	60.59 ± 1.37 (47)	20.37	25.2

Analysis of variance

Due to	D. F.	Sum of sq.	Mean sq.	Ratio observed
Treatment	7	27565.93	3937.99	23.85 **
Block	3	1846.16		
Error	21	3466.44	165.07	
Total	31	32878.53		

S. E. per plant 12.85

** Significant at 1 per cent level

TABLE X

Effect of different periods of chilling (mustard C 11)
(Unsplit chilled seeds dried for 16 days, sown October 9, 1939)

Period of chilling (weeks)	Vegetative period (days)	Earliness	
		Days	Percentage
Control	85.43 ± 3.16 (23)
2	77.59 ± 3.17 (22)	7.84	9.17
3	65.09 ± 5.19 (22)	20.34	23.81
5	68.25 ± 4.46 (24)	17.18	20.11
7	67.67 ± 3.82 (22)	17.76	20.79

Analysis of variance

Due to	D. F.	Sum of sq.	Mean sq.	Ratio observed
Treatment	4	6557.11	1639.27	7.46 **
Block	3	1139.73		
Error	12	2634.08	219.5	
Total	19	10330.92

S. E. per plant 14.8

** Significant at 1 per cent level

TABLE XI

Effect of different periods of chilling (mustard C 9)
(Unsplit chilled seeds dried for 16 days, sown October 9, 1939)

Period of chilling (weeks)	Vegetative period (days)	Earliness	
		Days	Percentage
Control	94.44 ± 4.8 (25)
2	80.35 ± 2.71 (30)	14.09	14.9
3	73.19 ± 4.29 (26)	21.25	22.5
10	65.32 ± 3.33 (28)	29.12	30.8
14	64.87 ± 3.37 (30)	29.57	31.3

Analysis of variance

Due to	D. F.	Sum of sq.	Mean sq.	Ratios observed
Treatment . . .	4	16152.65	4038.16	19.02 **
Block . . .	3	11583.78		
Error . . .	12	2547.75	212.31	
Total .	19	30284.18

S. E. per plant 14.56

** Significant at 1 per cent level

For trials in Cawnpore (Plate I, fig. 3) and in New Delhi, unsplit vernalized seeds chilled for 14 weeks were sent. The shortening of the vegetative period of plants from vernalized seeds of different strains of mustard observed in different stations is given in Table XII. It will be seen that irrespective of the region, vernalized unsplit seeds produced plants with shorter vegetative period, but that the earliness observed varied according to the strain of mustard. The differences observed in the percentage of shortening of the vegetative periods of the plants from the same batches of vernalized and untreated seeds but grown in different regions must obviously be due to the after-sowing environmental factors of the regions concerned.

TABLE XII

Vegetative periods of plants from control and vernalized seeds of different strains of mustard grown in different regions

Strain	Sowing date	Station	Vegetative period (days)	Earliness	
				Days	Percentage
Type 27 . .	2-10-39	Almora . .	C—80.96 V—60.59	20.37	25.2
	17-10-39	Cawnpore .	C—65.0 V—52.0	13.0	20.0
	21-10-39	New Delhi .	C—88.03 V—78.38	9.65	10.9
C 11 . . .	9-10-39	Almora . .	C—85.43 V—67.67	17.76	20.8
	17-10-39	Cawnpore .	C—48.0 V—41.0	7.0	14.6
C 9 . . .	9-10-39	Almora . .	C—94.44 V—64.87	29.57	31.3
	17-10-39	Cawnpore .	C—63.0 V—46.0	17.0	27.0

MUSTARD TYPE 27 FROM UNTREATED CONTROL AND VERNALIZED SEEDS



FIG. 1. Plants in pot 53 are from untreated control seeds and those in pot 41 are from unsplit seeds chilled for 50 days [Seeds sown May 15, 1939, photographed June 14, 1939, Almora]



FIG. 2. Plants in two pots in the middle are from control seeds ; those in two pots on the left, from seeds chilled for 8 weeks ; those in two pots on the right from seeds chilled for 6 weeks [Seeds sown July 11, 1938, Almora]



FIG. 3. Seeds sown August 11, 1939, photographed October 23, 1939, Cawnpore Agricultural Farm [Vernalized unsplit seeds used were chilled for 77 days and dried for 8 days (T. S. Sabnis)]



FIG. 1. Three sets of pots of mustard type 27 grown under different photoperiods.
Photographed on July 3, 1940



FIG. 2. The same pots on July 17, 1940



FIG. 3. One pot each from the three different sets showing similar vegetative periods of C-plants under 14 hrs photoperiods and V-plants under 10 hrs photoperiod. Photographed July 27, 1940

[Plants in left half of each pot are from maximally vernalized unsplit seeds, those on the right half, from untreated control seeds. Sown on June 6, 1940. Light treatment from June 11, 1940. Pots 38-41 had full day-length of 14 hrs, pots 42-45 had 11 hrs daylight for 3 weeks and full day-length afterwards, pots 46-49 had 8 hrs daylight for 3 weeks and full day-length afterwards.]

The effect of drying and storage on vernalized unsplit seeds

For environmental studies, the complications due to low-temperature requirements of the first phase of development can be eliminated by the use of vernalized seeds. With the limited facilities at our disposal, the problem of supply of strictly comparable vernalized seeds for different seasonal sowings at first appeared formidable, since it was not possible to arrange throughout the year strictly controlled similar low-temperature range, moisture supply and oxygen tension for chilling. But the fact that even incompletely vernalized unsplit seeds when dried and stored for 158 days were found to retain the induced vernalization (Experiment 6) suggested the possibility of using the same batch of vernalized unsplit seeds for different seasonal sowings. If the plants from the same batch of vernalized unsplit seeds would show similar vegetative periods in similar sowing dates of two successive years, it could be assumed that, (a) the induced vernalization had remained unimpaired for the period, and (b) any observed variations in the vegetative periods in the intermediate sowings were due to changes in after-sowing temperature and day-length of the season. Therefore a long series of sowings from the same batch of vernalized seeds was undertaken, to find out the period for which vernalized unsplit seeds when dried and stored would retain the induced vernalization unimpaired.

EXPERIMENT 11

A batch of mustard seeds (Type 27) was chilled for 167 days, much longer than was necessary to induce maximum vernalization. The period was purposely prolonged to repeat the observation of Experiment 6, to find out whether prolonged chilling would be injurious to unsplit seeds. The seeds were placed in the moist-chamber of the Electrolux on October 18, 1938 and were removed on April 3, 1939. After being dried for three days at room temperature, the unsplit chilled seeds were put in cold storage in a sealed bottle. All sowings from this batch of vernalized seeds, along with the controls, were made in pots kept in the glass-house. A daily record of the maximum and minimum temperature of the glass-house was kept. The curve of the seasonal day-length of Almora was obtained from Dr L. A. Ramdas, of the India Meteorological Department, Poona. Readings of the dry-and-wet-bulb thermometer kept in the glass-house were taken every afternoon. The results of the 20 sowings spread over the period from April 24, 1939, to March 28, 1941, are summarized in Table XIII. For convenience the different sowings on similar dates of two successive years are grouped together. It will be seen that the total period of drying of vernalized unsplit seeds in sowings Nos. 1 and 9 were 21 and 387 days, respectively, and yet the observed vegetative periods of the plants were remarkably similar. The vegetative periods of plants from control seeds in these two sowings were also very similar. Therefore, it may be concluded that drying of vernalized unsplit seeds for a period of 387 days did not induce any devernalization.

But from the comparison of the data from sowings Nos. 2 and 11 of June 26, 1939, and of June 27, 1940, no definite conclusion about the condition of the V-seeds seems to be justified, in spite of the fact that the plants from these seeds had very similar vegetative periods. For a marked shortening of the

vegetative period of plants from C-seeds was observed in sowings of 1940 which obviously must have been due to more favourable after-sowing environmental conditions of the year. The lack of any observable effect of favourable after-sowing environmental factors upon the vegetative period of plants from V-seeds might very possibly have been due to slight devernization resulting from prolonged drying, or it might have been due to the fact that the vegetative period of 29.9 days observed in 1939 was already the minimum for plants from these seeds, and therefore no further diminution could be expected. Therefore, in the next sowing of this series an independent estimate was sought about the condition of this particular batch of V-seed by sowing on the same date with them seeds from another batch of vernalized unsplit seeds chilled for 365 days but dried for seven days only. The result of sowing No. 12 indicate that the condition of the V-seeds chilled for 167 days and dried for 462 days was similar to that of seeds chilled for 365 days and dried only for seven days. In other words, the vernalization induced in seeds remained unimpaired even when the seeds were dried for 462 days. A similar test with seeds chilled for 365 days was undertaken in sowing No. 18 with similar verification. In all sowings under similar conditions, we have not found any batch of chilled unsplit seeds, including those chilled for 365 days, which produced plants with shorter vegetative period than that of plants from seeds chilled for 167 days.

To find out whether the capacity of unsplit vernalized seeds to withstand drying was equally true of other batches of maximally vernalized seeds chilled for a period shorter than 167 days, unsplit seeds from batch (a) chilled for 65 days was sown on June 14, 1941, together with batch (b) chilled for 365 days for comparison. On that date the period of drying and storage of a-seeds was 765 days and of b-seeds 348 days. The observed vegetative periods were 31.75 ± 0.82 days (mean of 16 a-plants) and 31.14 ± 0.71 days (mean of 7 b-plants). Thus, it is evident that maximally vernalized unsplit seeds of mustard Type 27 irrespective of period of chilling can withstand drying and storage and still retain the effect of vernalization for at least 765 days.

From the data given in Table XIII it can be concluded that : (1) in all the different sowings plants from the same batch of vernalized seeds flowered significantly earlier than those from untreated control seeds ; (2) an annual cyclic variation of the vegetative periods of plants alike from vernalized and control seeds, due to differing after-sowing environmental conditions of the seasons, can normally be expected ; (3) similar after-sowing environmental conditions affect the vegetative periods of C- and V-plants differently, since the shortest vegetative period of approximately 41 days observed in C-plants was in sowings of April and May of 1939 and 1940, while in the case of V-plants the shortest period of about 31 days was observed in sowings of April to August of both 1939 and 1940. The longest vegetative periods of C- and V-plants, however, were observed in sowings of October—December ; (4) maximally vernalized unsplit seeds of mustard when dried and stored for a period of 72 days retain the induced vernalization unimpaired. This last conclusion offered the most convenient solution of the problem of supply of strictly comparable vernalized seeds for environmental studies, for seeds from a batch of maximally vernalized seeds can be used for sowings spread over a period

TABLE XIII

Vegetative period of plants from seeds chilled for 167 days and sown at different seasons of two successive years

(Mustard Type 27)

No.	Sowing date	Vegetative periods (days)		Drying (days)	Earliness in V-plants	
		Control	Vernalized		Days	Per cent
	<i>April</i>					
1	24, 1939	41.5 ± 1.95 (4)	31.9 ± 0.54 (8)	21	9.6	23.1
9	24, 1940	41.03 ± 0.65 (15)	30.53 ± 0.41 (15)	387	10.5	25.6
	<i>May</i>					
10	22, 1940	41.3 ± 0.73 (12)	30.0 ± 0.72 (10)	415	11.3	27.3
	<i>June</i>					
2	27, 1939	54.3 ± 0.58 (3)	29.9 ± 1.48 (5)	85	24.4	44.9
11	29, 1940	46.75 ± 0.92 (8)	29.5 ± 0.49 (11)	453	17.25	36.9
	<i>July</i>					
12	8, 1940	47.18 ± 0.85 (11)	31.75 ± 0.64 (12)	462	15.43	32.7
	8, 1940	(Chilled 365 days)	31.00 ± 0.92 (7)	7	16.18	34.3)†
	<i>August</i>					
3	11, 1939	59.9 ± 2.06 (10)	23.9 ± 0.79 (8)	130	31.0	51.7
13	12, 1940	52.6 ± 1.28 (8)	32.1 ± 0.76 (6)	497	20.5	38.9
	<i>September</i>					
14	26, 1940	60.77 ± 1.11 (13)	37.6 ± 0.58 (11)	542	23.17	38.1
	<i>October</i>					
4	11, 1939	82.0 ± 1.6 (8)	50.0 ± 2.42 (8)	191	32.0	39.0
15	26, 1940	99.2 ± 1.2 (5)	80.2 ± 1.53 (8)	572	19.0	19.2
	<i>November</i>					
16	26, 1940	90.5 ± 0.42 (9)	86.1 ± 0.37 (8)	603	4.4	4.9
	<i>December</i>					
5	16, 1939	82.75 ± 0.23 (8)	73.12 ± 0.52 (8)	257	4.63	5.6
17	26, 1940	74.22 ± 0.14 (9)	71.55 ± 0.32 (9)	633	2.67	3.6
	26, 1940	(Chilled 365 days)	71.8 ± 0.59 (5)	178	2.42	3.2)†
	<i>January</i>					
6	29, 1940	62.6 ± 0.5 (17)	59.07 ± 0.32 (13)	301	3.53	5.6
18	27, 1941	56.0 ± 0.47 (8)	50.62 ± 0.23 (8)	665	5.38	9.6
	<i>February</i>					
7	16, 1940	53.23 ± 0.37 (7)	43.75 ± 0.46 (8)	319	4.53	8.5
19	25, 1941	46.58 ± 0.36 (12)	42.0 ± 1.0 (6)	694	4.58	9.8
	<i>March</i>					
8	20, 1940	45.25 ± 2.58 (4)	37.5 ± 1.48 (4)	352	7.75	17.1
20	23, 1941	43.83 ± 1.32 (6)	34.87 ± 0.57 (8)	725	8.96	20.4

† These sowings were undertaken to obtain independent index of the condition of the batch of seeds chilled for 167 days.

of two years. The other important consequence of this finding is that maximally vernalized unsplit seeds can be used to determine the degree of vernalization induced in unsplit seeds during the process of chilling—for which no other reliable index has so far been discovered. For instance, if in any simultaneous sowing of maximally vernalized unsplit seeds together with samples of any other batch or batches of seeds then in process of chilling, the observed vegetative periods of the plants are found to be similar, then the seeds tested may be considered maximally vernalized. If, on the other hand, the vegetative periods of the sample seeds are longer, then the chilling process should be continued till in later similar sowings the seeds produce plants with similar vegetative periods.

The purpose of the above experiment was to explore the possibility of maintaining the induced vernalization in unsplit seeds unimpaired for the longest possible period, and therefore the seeds were stored, as already stated, in the chilling-cabinet. This extra precaution of cold storage has since been found unnecessary. For it has been found that vernalized unsplit mustard seeds can be subjected before sowing to high temperature, without any devernalization. In a sowing of April 24, 1940, V-seeds kept throughout in cold storage 384 days produced plants with a vegetative period of 30.53 ± 0.41 days (mean of 15 plants), while seeds from the same sample which were subjected to $30^{\circ}-2^{\circ}\text{C}$. for 39 days before sowing produced plants with similar vegetative period of 30.3 ± 0.58 days (mean of 13 plants). A similar sowing undertaken on August 13, 1941, from a sample (L) of a batch of seeds chilled for 167 days and kept in cold storage for 863 days and from another sample (H) which, after being kept in cold storage for 347 days, was kept at room temperature for 516 days. The observed vegetative periods were 33.0 ± 0.88 days (mean of 10 plants) for L-plants and 31.14 ± 0.67 days (mean of 7 plants) for H-plants. The difference of 1.86 days is not statistically significant. This indicates that storage at room temperature for over 73 weeks does not induce any devernalization.

Temperature and photoperiod requirement of second phase of development

It has been shown by Gilbert [1926], Purvis [1934], Steinberg and Garner [1936] and others that the factor of temperature must be taken into careful consideration in all photoperiodic studies. Lacking facilities for automatic control and maintenance of different combinations of temperature and photoperiod, we have utilized the natural variations in the seasonal complements of temperature and day-length to observe the effect of after-sowing environmental factors. Since the seasonal temperature and day-length vary in a similar way, i.e. high temperature is associated with long days and low temperature with short days, the data obtained from different seasonal sowings give the resultant effect of these factors varying in a similar way. Therefore, to determine the optimum after-sowing temperature and photoperiod for mustard it was necessary to observe the vegetative periods of plants grown either under (i) similar temperature ranges, or (ii) similar photoperiods throughout the year. The second alternative was adopted for the following series of observations, since the arrangements for subjecting potted plants to similar effective photoperiods throughout can be easily devised.

Tincker [1925] found that an intensity of 5 foot-candles of visible radiation is adequate for prolonging the day-length for photoperiodic reactions.

China aster Withrow and Benedict [1936] observed definite photoperiodic effect, when the intensity of the supplementary light was 0.3 foot candle and as low as 0.1 foot candle. The same authors observed that the orange and the red end of the spectrum caused the most marked photoperiodic response. Therefore, to supplement the seasonal day-length for increased photoperiod, a hanging Petromax kerosene lamp (500 c.p.) of the type commonly used as a street light, suspended from the ceiling of an open verandah, was adopted as a convenient arrangement. Except for the winter months, it was found necessary to protect the seedlings against the insects—which the bright light invariably attracted—by a mosquito curtain hung from a fine wire-net frame 4ft. \times 4ft. attached to the enamel reflector of the hanging lamp. Despite the removal of all obstructions against free circulation of air in the verandah, the temperature rise from 2° to 5°C. under the lamp could not be overcome in this arrangement. For subjecting potted plants to photoperiods shorter than the seasonal day-length, the required number of hours of the morning light was cut off by keeping the pots in a well-ventilated dark chamber constructed in the glass-house.

EXPERIMENT 12

For this experiment 10 different sowings from April, 1940, to March, 1941, were undertaken. Control and maximally vernalized unsplit seeds were sown in the two halves of several pots used for each sowing. Different sets of pots were subjected to different photoperiods. At the beginning of the light treatment, four plants were kept in each pot—two from control and two from vernalized seeds. Towards the end of the experiment, some of the plants died, and therefore in later sowings of this series the original number of plants was increased either by increasing the number of pots for each light treatment from three to four or, when the available bench space in the glass-house was inadequate, by increasing the number of plants from four to six in each pot.

The pots containing the seedlings which were subjected to supplementary artificial light were daily removed after sunset to the open verandah and were kept on a wooden platform under the hanging Petromax lantern for the required periods, after which they were brought back to the open benches of the glass-house. For photoperiods shorter than day-length, sets of pots were removed from the open benches in the glass-house to the dark chamber after sunset and were kept there till the required time in the morning, after which they were placed on the open benches in the glass-house. A control batch of seedlings, kept throughout the experiment on the open benches, was subjected to the normal day-length of the season. The position of the pots was changed every few days to secure as far as possible similar light conditions. Since all seeds were sown on the same date, it is assumed that plants in each series were subjected to a similar seasonal temperature range. The temperature variation during the light treatment did not, as will be seen later, produce any appreciable complication.

The results obtained from this series of sowings are given in Table XIV. In the first sowing of April 2, 1940, plants were subjected to photoperiods of normal day-length of 13 hours, day-length plus artificial light for three hours (16 hours), and day-length diminished by three hours (10 hours). The

light treatment began on April 9, and after three weeks' treatment, flower buds were distinctly visible on all plants from vernalized seeds subjected to photoperiods of 13 hours and 16 hours, and the average period for the opening of the first flower in all these plants was very similar, being 34.18 days and 34.8 days, respectively. Therefore it was tentatively assumed that for plants from vernalized seeds of mustard Type 27 a photoperiodic treatment of 13 hours for three weeks was not below the optimum, and in the second sowing when the normal day-length was above 13 hours no supplementary artificial light was used. The assumption that photoperiods longer than 13 hours do not induce any further shortening of the vegetative period was verified from the subsequent sowings Nos. 3, 7 and 8. In all sowings the period of light treatment was similar, i.e. three weeks only.

TABLE XIV

Vegetative periods of C- and V-plants under different temperatures and photoperiods

(N, Normal day-length ; S, date of sowing ; L, light treatment)

		Photo-period			
No.	Date	(1)	(2)	(3)	(4)
1940					
1	S 2/4	16 hours	13 hours N	10 hours	
	L 9/4	C—39.8±0.66 (5) V—34.8±1.04 (5)	C—42.4±0.82 (5) V—34.2±0.65 (6)	C—52.2±1.36 (3) V—41.0±0 (3)
2	S 7/6	14 hours N		11 hours	8 hours
	L 11/6	C—48.7±0.42 (11) V—32.4±0.7 (12)	55.2±0.35 (9) 40.8±0.89 (11)	62.4±0.67 (7) 50.1±1.16 (8)
3	S 12/8	14 hours	13 hours N	10 hours	7 hours
	L 17/8	C—53.0±1.27 (8) V—31.8±0.71 (7)	52.6±1.28 (8) 32.1±0.76 (6)	63.9±1.9 (7) 39.5±1.34 (4)	68.0±1.0 (4) 51.6±1.32 (6)
4	S 26/9	13.5 hours		11.5 hours N	9.5 hours
	L 1/10	C—55.8±1.46 (11) V—37.1±0.82 (9)	60.7±1.11 (13) 37.6±0.58 (11)	73.4±1.83 (11) 47.3±1.74 (10)
5	S 26/10		13 hours	10.75 hours N	9.25 hours
	L 3/11	C—97.6±1.45 (5) V—71.4±1.07 (8)	99.2±1.3 (5) 80.0±1.53 (8)	106.4±1.53 (9) 85.0±1.11 (9)
6	S 26/11	15 hours		10.25 hours N	9.25 hours
	L 6/12	C—89.8±0.3 (9) V—84.0±0.25 (8)	90.5±0.42 (9) 86.1±0.37 (8)	91.0±0.31 (8) 86.2±0.36 (9)
7	S 26/12	15.25 hours	13.25 hours	10.75 hours N	9.25 hours
	L 15/1/1941	C—72.7±0.46 (8) V—69.2±0.44 (9)	73.1±0.33 (9) 70.5±0.5 (8)	74.2±0.14 (9) 71.5±0.32 (9)	74.2±0.62 (9) 70.3±0.31 (9)
1941					
8	S 27/1	15.25 hours	13.25 hours	11.25 hours N	9.25 hours
	L 8/2	C—53.3±0.27 (9) V—49.2±0.24 (9)	53.8±0.3 (9) 49.9±0.29 (9)	56.0±0.47 (8) 50.6±0.28 (8)	57.8±0.63 (9) 51.9±0.73 (7)
9	S 25/2	15.25 hours		12 hours N	8.5 hours
	L 9/3	C—42.0±0.35 (9) V—37.5±0.28 (13)	46.6±0.36 (12) 42.0±1.0 (6)	54.9±1.02 (7) 44.0±0.76 (7)
10	S 28/3	15.5 hours		12.5 hours N	9.5 hours
	L 6/4	C—40.9±0.59 (8) V—34.0±0.54 (8)	43.8±1.32 (6) 34.9±0.57 (8)	53.6±1.7 (5) 40.4±1.16 (7)

Obviously from the nature of the data, conclusions of only a qualitative nature are justified, since of the several factors involved in these experiments, only the nature of the seeds used and actual periods for which the day-length of the season were supplemented or diminished are known. It was not possible to obtain accurate data of even the effective day-lengths which were supplemented or diminished. With regard to the temperature, only the maximum day temperature and the minimum night temperature of the glass-house were recorded, and from these no idea could be obtained as to the actual duration of the different temperatures to which the plants were subjected throughout the 24-hour period. Neither was it possible to obtain a record of humidity variation of the glass-house, beyond the afternoon records of the readings of the dry-and-wet-bulb thermometer.

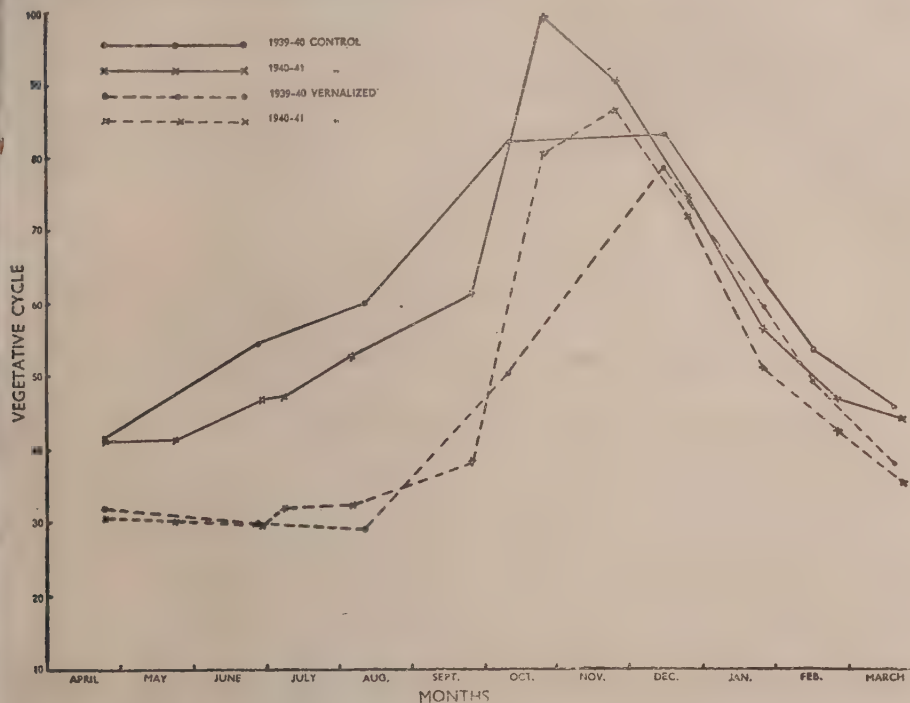


FIG. 1. Vegetative periods (days) of plants from the same batches of control and maximally vernalized unsplit seeds (mustard Type 27), in different seasonal sowings of 1939-40 and 1940-41

Despite these limitations, the following conclusions seem to be justified: (1) Under all similar temperatures and photoperiods so far studied plants from vernalized seeds of mustard Type 27 flower significantly earlier than those from the untreated controls (Figs. 2 and 3). (2) No critical photoperiod is discoverable for mustard Type 27, since flowers are produced from plants which have been subjected to a photoperiod of 16 hours in April sowing (treatment 1) as well as $9\frac{1}{2}$ hours for the first three weeks in October sowing (treatment 4) and afterwards from November 25, 1940, to January 19,

1941, to normal day-lengths of $10\frac{1}{2}$ - $10\frac{1}{4}$ - $10\frac{1}{2}$ hours. But under all temperature ranges prevailing in Almora, except the limiting one during sowing VI,

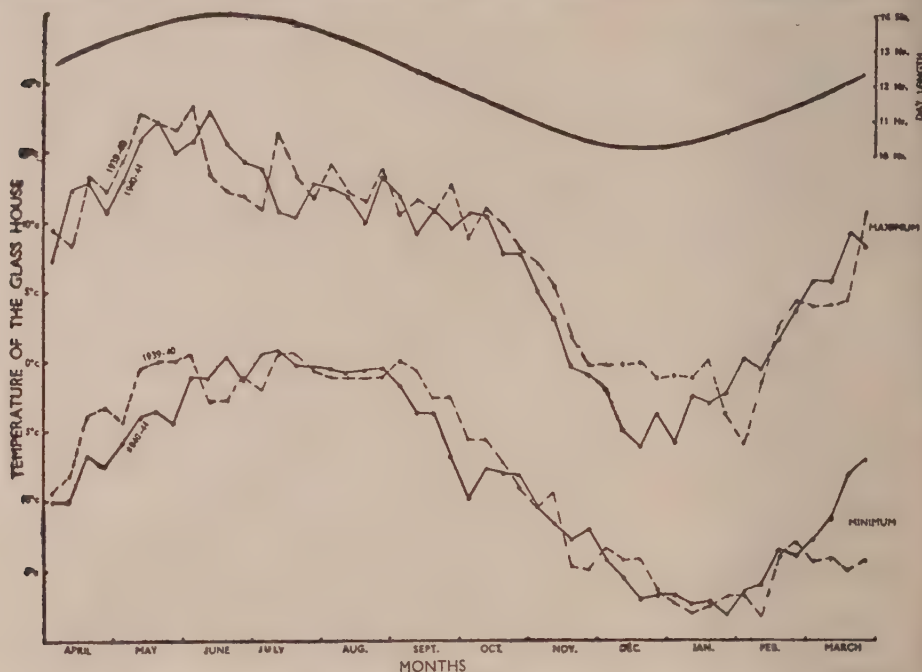


FIG. 2. Weekly average maximum and minimum temperatures of the glass-house (1939-40 and 1940-41) and the day-length of Almora

of November 26, 1940, the observed vegetative period progressively diminished as the photoperiod was increased up to 13 hours. Increase in photoperiod beyond 13 hours—16 hours in April, 14 hours in August, 15 hours in December, $15\frac{1}{2}$ hours in January and February—did not produce any further shortening of the vegetative period. From this it can be concluded that the optimum photoperiod for the second phase of development of mustard Type 27 is not more than 13 hours. Incidentally it is shown that a temperature rise of 2° - 5°C . during supplementary light treatment for two to three hours produces no significant difference as far as the vegetative period is concerned. (3) The increased vegetative periods observed in winter sowings are due mainly to the prevailing low temperature and not to diminished day-length. For it will be seen that in sowing 1 (when the average maximum day temperature was 31°C .) the observed vegetative periods with photoperiods of 13 hours for three weeks were 34.18 days for V-plants and 42.4 days for C-plants, but in November sowing (No. 6) the vegetative periods observed with a photoperiod of 15 hours for three weeks (when the average maximum day temperature varied from 20° to 15°C .) were 84 days for V-plants and 89.8 days for C-plants. (4) The optimum day-temperature of the second phase of development of mustard Type 27 appears to be 30°C ., for under all similar photoperiods in all sowings from April 24 to August 12, both in 1939 and 1940,

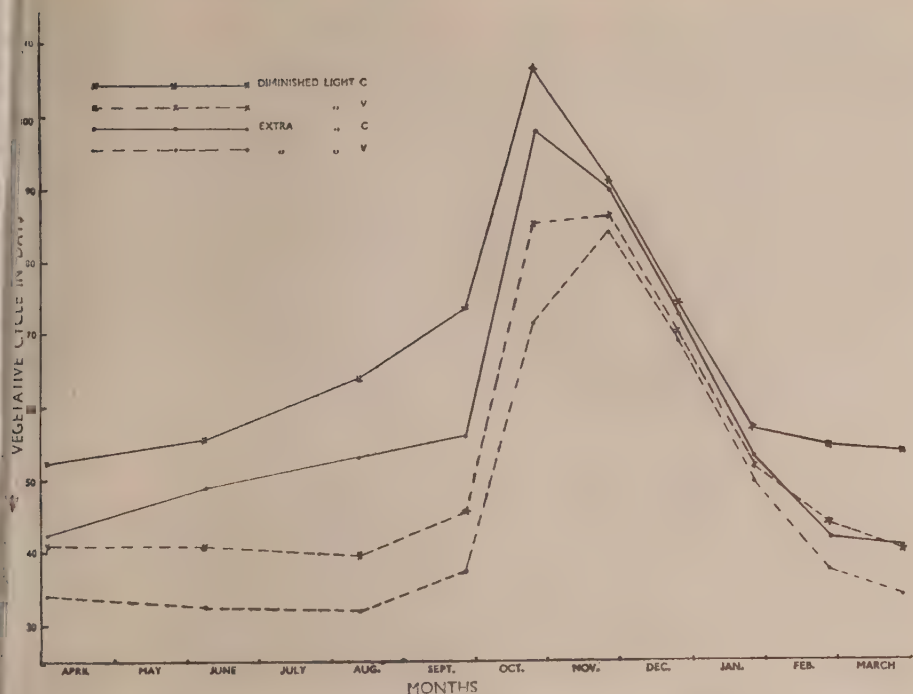


FIG. 3. Vegetative periods of plants from the same batches of control and maximally vernalized unsplit seeds (mustard Type 27) under photoperiods longer and shorter than seasonal day-length

(Tables XIII and XIV) when the maximum temperature varied from 30° to $38^{\circ}\text{C}.$, the observed vegetative periods of V-plants were similar and minimum. When the maximum day temperature was below $30^{\circ}\text{C}.$, however, the vegetative periods were found to increase progressively. (5) The differential response of C- and V-plants to similar after-sowing environmental factors observed in seasonal sowings of 1939-40 and 1940-41 can be explained if the minimum night temperature is taken into consideration. In sowings of June, July and August (Tables XIII and XIV), when the natural seasonal complements of day temperature and day-length were optimum (Fig. 2), the vegetative period of V-plants were remarkably similar, while those of C-plants were found to increase steadily from June sowings onwards. From the minimum temperature curve of the glass-house, it will be seen that from mid-April to the first week of June, the night temperature is lower than from the last week of June to the end of August. Furthermore, the period from April till the monsoon starts—about the middle of June—is the driest one in Almora, and the temperature of the moist soil in pots is generally $3^{\circ}\text{--}5^{\circ}\text{C}.$ lower than the recorded air temperature of the glass-house. The average minimum night temperature of the air for five days following April 24, 1940, was $13^{\circ}\text{C}.$, and for the five days following May 22, it was $16.5^{\circ}\text{C}.$, while the minimum night temperature for five days following June 7, 1940, was $20^{\circ}\text{C}.$ The prevailing night soil temperature in Almora from late April

to the beginning of June is thus of the order of the temperature required for vernalization (Experiment 2), and it is reasonable to suppose that in this region in sowings of April and May partial natural chilling takes place in the case of untreated controls, at least to a greater extent than during the hotter nights of June, July and August. It was shown in our preliminary report [Sen and Chakravarti, 1938] that sprouted mustard seeds of Type 27 chilled for only four days, produced plants in the September sowing of 1937 which flowered nine days earlier than those from the control sprouted seeds. In the case of plants from maximally vernalized seeds, however, neither the cooler nights of April and May, nor the hotter nights from June to August, affect the vegetative period. The verification of the above assumption is seen in the minimum difference in the vegetative periods of C- and V-plants, in sowings of November and December, where the advantage of the V-plants (pre-supply of low temperature) is reduced to a minimum by the natural winter temperature of this region.

DISCUSSION AND CONCLUSIONS

From the results of the experiments described, it is evident that all the five strains of mustard—Type 27, C 11, C 9, yellow sarson, and *raya* O.B I—respond to vernalization, and chilled unsplit seeds of mustard show all the characteristics of vernalized seeds. For instance, in the case of mustard Type 27, which has been used for most of the experiments, unsplit chilled seeds produce plants which flower earlier than plants from untreated seeds, the vernalization induced in unsplit chilled seeds increases with increased dose of chilling until, under optimum conditions, maximum vernalization is attained by chilling for six weeks. Prolonged chilling up to 365 days neither induces any higher degree of vernalization in unsplit seeds nor any devernalization. These observations are in accord with the findings of Lojkin [1936] in connection with vernalization of winter wheat. The same author observed that under field conditions the percentage of germination of vernalized wheat was lower than for untreated seeds, and this has also been observed by us (unpublished data) in the case of vernalized winter wheat. But in the case of vernalized unsplit mustard seeds, the germination has been found to be similar to that of control seeds [Sen and Chakravarti, 1938]. From Tables IX, X and XI, in which are given the data obtained from normal seasonal sowing under field conditions, it will be seen that the earliness in flowering of plants from maximally vernalized mustard seeds was 17.76 days for C 11, 25.4 days for Type 27 and 29.57 days for C 9. In the case of mustard Type 27, plants from vernalized seeds have been found to flower earlier under all combinations of after-sowing temperature ranges and photoperiods studied (Tables XIII and XIV). Thus, a definitely earlier harvest can be insured by the use of vernalized unsplit seeds of mustard, which also have the advantage that they are capable of being stored for over two years without deterioration.

Yield

For practical agriculture, the yield and the quality of the crop are as important as earlier harvest, if not more important. Whether earlier harvest obtained from vernalized mustard seeds can be associated with higher yield and better quality of crop, under otherwise similar cultural conditions, depends

the environmental factors prevailing during the period of seed-setting and seed-ripening. In the case of wheat, Kirichenko [1934] observed that, making the photoperiodic requirement of the third phase, seeds would not set, as the pollen became sterile. Ali Mohammad and Ahmad [1940] have shown that the oil content of mustard depends, among other factors, on the temperature during seed formation. In the case of an insect-pollinated crop, the population of the pollinating insects during the period of full bloom is also an important factor which determines the yield. Furthermore, it should be realized that extreme shortening of the life-cycle under optimum environmental conditions would in all probability produce ephemeral plants and obviously their yield would be considerably lower than normal. For instance, in a small field-plot sowings of June, 1938, the total period from sowing to harvesting of mustard Type 27 was 99 days for V-plants, and the yield was 452 ± 0.48 grams (mean of 38 plants) per plant. In sowings of October, 1939, when the V-plants took 208 days to complete the life-cycle, the average yield per plant was 22.9 ± 1.45 grams (mean of 91 plants).

To derive the maximum advantage from vernalized seeds, new optimal sowing dates should be discovered for different regions, as Whyte [1939] has already pointed out. Since the environmental requirements of the different phases of development of a crop are not identical, and yield and quality of the crop are the final expression of the life-cycle, higher yield and better quality of crop can be expected from vernalized seeds if the facility offered by early flowering can be utilized to secure (preferentially) for V-plants better environmental factors for the completion of the life-cycle. Greater yield can also be expected if, by earlier harvest, the hazards of pests, drought, excessive rain, frost or snowfall can be avoided, or at least partially mitigated. Two cases may be cited, one of mitigation of caterpillar injury, the other, of greater damage resulting from snowfall, observed by us in connection with V-plants. In our first outdoor sowing of June 4, 1938 (Experiment 2) yield per plant was recorded. The average yield per V-plant was 4.55 grams (mean of 38 plants) and per C-plant, 1.98 grams (average of 25 plants), but this difference was not statistically significant; the higher average of V-plants was in reality due to the differential damage caused by caterpillar attack. The less advanced C-plants with their softer tissue system were more severely damaged than the taller V-plants, and some of the former were completely destroyed. In a small field-plot sowing of October 6, 1939, with six replications each of control and vernalized seeds, the yield per plant was again recorded. The yield observed per V-plant was 22.9 grams (mean of 91 plants) and 27.94 grams (mean of 95 plants) for C-plants. In this case also, though the observed difference was not statistically significant, the variation can be explained by the fact that on February 6 and 10, 1940, during the full-bloom period of the V-plants, which flowered 28.89 days earlier than the C-plants, two heavy snowfalls occurred, causing greater damage to the V-plants than to the less advanced control plants. But in our preliminary observations with pot-culture plants grown in the protected environment of a glass-house, the yield observed per V-plant has been found to be greater than that of C-plants, and this alike in off-season sowings of July and seasonal sowings of October. In sowings of July 11, 1938, where seeds chilled for different periods were used, the records of the following four characters of each plant were

taken : (i) time of opening of flowers (Table II) ; (ii) time of completion of flowering ; (iii) time of maturity ; (iv) yield per plant. The results of the statistical analysis of the data submitted to Prof. P. C. Mahalanobis of the Statistical Institute, Calcutta, show that in all these four characters V-plants had the advantage, i.e. compared with C-plants, V-plants flowered earlier flowering was completed earlier, seeds matured earlier and their weight per plant was greater. Thus, for example, the mean yield per C-plant was 0.49 gram and per V-plant (seeds chilled for six weeks) 1.025 grams, a difference which is statistically significant at 1 per cent level. In sowings of October 18, 1938, the observed yield (mean of 11 plants) per C-plant was 0.96 gram, while the yield (mean of eight plants) per V-plant was 1.68 grams, a difference significant at 5 per cent level. But from these data no definite conclusion is justified regarding yield of V-plants under field conditions. Therefore, pending the results of experiments now in progress to determine the optimum environmental requirements of the third phase and optimum temperature for seed-ripening, systematic investigation regarding the possibility of associating earlier harvest with higher yield and better quality of crop from vernalized seeds has been postponed.

With reference to the effect of drying and storage of vernalized seeds, Lojkin [1936] found that vernalized seeds of winter wheat when air-dried for four weeks at 1° and 15°C. are partially or completely devernalized. Gregory and Purvis [1938] found that when maximally vernalized seeds of winter rye are dried, the process of devernalization sets in after six weeks, and in course of 20 weeks complete devernalization takes place. Purvis and Gregory [1937] in explaining this devernalization by drying in terms of a suggested scheme of vernalization maintain that drying induces a reversal of the reaction which produces the substance responsible for early flowering in plants from vernalized embryos. But it will be seen from Experiment 11 that vernalized unsplit seeds of mustard can be dried for a period of 863 days without any observable devernalization. Therefore it can be concluded that drying even at room temperature does not devernalyze unsplit vernalized seeds of mustard. The contradictory effects of drying on vernalized seeds of mustard and of wheat and rye may be due either to the difference in the nature of the embryos concerned, or to the different stages of growth of the embryo of the vernalized seeds of mustard and of wheat and rye. The embryos of the vernalized seeds of wheat and rye developed to the seedling stage, while obviously in the case of vernalized unsplit seeds of mustard the growth of the embryo is confined within the elastic limit of the seed-coat. That the different effects of drying are not due to the types of the embryos concerned but to the stages of their development during the period of low-temperature treatment is suggested from the fact that in the case of rye also, when the developing embryo is chilled in the ear, no devernalization takes place when these seeds ripen and become dormant [Gregory and Purvis, 1938]. In natural vernalization of the seeds, the growth of the embryo is limited within the confines of the testa, which is also the case with chilled unsplit mustard seeds. Therefore it seems reasonable to conclude that so long as the growth of the embryo is confined within the elastic limit of the testa, it can retain the effect of chilling unimpaired for long periods. Investigations are in progress to find out the specific protective character of the seed-coat.

Gregory and Purvis [1938] have shown that the capacity of pre-chilled seeds to produce plants with shorter vegetative period is not due to delayed germination but to the specific effect of low temperature on the embryo, like during the period of its development during seed formation and when the dormant embryo is activated. In our experiments with mustard Type 27, earliness in flowering has been observed in plants both from chilled unsplit seeds as well as from unchilled seeds which developed during the winter months (Experiment 9). This natural vernalization, at least partial, induced by the prevailing low temperature during the period of seed ripening suggests interesting possibilities for vernalization. It is a common experience in the tropics that imported seeds of some of the winter annuals from colder climates give very good results, but fail to produce seeds as good as those of the parent stock. If the cause of the seed deterioration be mainly due to prevailing high temperature during the period of seed-ripening in the tropics then this defect could be corrected by shortening the vegetative period through the use of V-seeds, provided the crop responds to vernalization.

Plants from untreated seeds of mustard Type 27 have been found to flower under all the different seasonal complements of temperature and day-length prevailing in Almora. In our glass-house the maximum day temperature during the year varies from 40° to 10°C . and the minimum night temperature from 22° to 1°C . The day-lengths vary from 10.2 hours to 14 hours. In all sowings, under similar environmental conditions, V-plants flower significantly earlier than C-plants, yet it would appear that low temperature is not an obligatory factor for inflorescence of mustard Type 27. For it will be seen from Table XIII that, when the minimum night temperature averaged 20°C . (Fig. 2) during the months of June and July, 1940, plants from untreated seeds flowered in 46.75 and 47.18 days, respectively (Nos. 11 and 12), which was only about half the periods required for flowering by similar plants in winter sowing of October and November, namely, 99.2 and 90.5 days, respectively (Nos. 16 and 17). That the shorter vegetative period observed in summer is not due primarily to optimum photoperiod, but to temperature, will be seen from the results of sowing No. 1 (Table XIV), where the observed vegetative period of C-plants grown under 10 hours photoperiod (shorter than Almora winter day-length) for three weeks and subsequently under normal day-length of 13 hours was 52.2 days, while in sowing No. 3 under exactly similar photoperiods the observed vegetative period was 69.9 days. Therefore it can be concluded that for mustard Type 27, in spite of the fact that there is no specific low-temperature requirement of the first phase, low temperature, whether pre-supplied to the embryo during seed formation or to the embryo of the unsplit seeds or of sprouted seeds, will shorten the vegetative period of the plants. The quantitative nature of the effect of low temperature on the development of mustard is proved by the fact that the vernalization induced increases up to a maximum with increased dose of chilling (Experiment 2).

In the case of winter rye, Purvis and Gregory [1937] found that seedlings subjected to decreased photoperiod for six weeks at the initial stage will advance to the reproductive stage earlier. From the data given in Table XIV, it will be seen that increased photoperiod for the first three weeks will shorten the vegetative period of mustard Type 27. In sowing 2 of June 6, 1940,

(when the day temperature was optimum and the minimum night temperature was about 20°C.), the V-plants flowered earlier than the corresponding C-plants under all the three photoperiodic treatments. As the photoperiods were shortened from the normal day-length of 14 hours, the vegetative periods of both V- and C-plants increased. The vegetative periods of plants subjected to eight hours photoperiod for the first three weeks and subsequently to normal day-length of 14 hours were 62.4 days for C-plants and 50.1 days for V-plants, while the vegetative period of C-plants subjected throughout to normal day-length of 14 hours was 48.7 days, which was similar to that of V-plants (50.1 days) subjected to a shorter photoperiod (Plate II), for the observed difference of 1.4 days is not statistically significant. The C-plants under 14 hours photoperiod were subjected to the minimum temperature which averaged 20°C. throughout their first and second phases, yet they flowered at the same time as the V-plants subjected to diminished photoperiod. Thus it can be concluded that a similar shortening of the vegetative period can be obtained either by pre-supply of low temperature to the embryo or by subjecting seedlings to increased photoperiod. From the observations recorded it would appear that the original concepts of Lysenko's theory of phasic development of annual seed crops are not applicable to mustard Type 27, either in regard to the obligatory qualitative nature of the changes produced by low temperature during the first phase, or the strict dependence of each phase on the completion of the preceding phase.

SUMMARY

Vernalization response of different strains of mustard—Type 27 from New Delhi, Types C 11 and C 9 from Cawnpore, *raya* O.B/I and yellow *sarson* from Lyallpur—has been observed. Most of the environmental studies were, however, carried out with mustard Type 27. Simple techniques, without facilities of electric supply, for vernalization of seeds and determination of after-sowing optimum temperature and photoperiod have been described. From the observed vegetative periods of plants grown in pots as well as in small field plots the following conclusions have been reached :—

1. All the five strains of mustard respond to vernalization, i.e. plants from vernalized seeds flower earlier than those from untreated seeds.
2. For a similar dose of chilling the vernalization response of different strains of mustard varies.
3. Seeds which sprout as also those which remain unsplit, during the period of chilling are vernalized; but for the same dose of chilling, earliness observed in plants from sprouted chilled seeds is greater. The degree of vernalization induced increases to a maximum with increased dose of chilling. Under optimum chilling conditions maximum vernalization is induced in unsplit chilled seeds of mustard Type 27 in six weeks; further prolongation of chilling up to 365 days does not induce any higher degree of vernalization, nor any devernialization.
4. While drying is fatal for sprouted chilled seeds of mustard, chilled unsplit seeds can be dried, and drying does not affect subsequent germination.
5. The observed vegetative periods of plants (Type 27) from progenies of seeds vernalized for three successive generations do not indicate any transmission of the effect of vernalization to the offspring.

6. When growth of the embryo is confined within the elastic limit of the seed-coat, the chilled seeds can be dried and stored for long periods (83 days so far observed) without any resultant devernalization.

7. Under all similar after-sowing temperature range and photoperiods so studied, plants from vernalized unsplit seeds flower earlier. Thus an earlier harvest can be obtained by the use of vernalized unsplit seeds. The possibilities of associating yield with earlier harvest are discussed.

8. Mustard Type 27 has no obligatory low-temperature requirement in the first phase, for plants from untreated seeds will flower even when the minimum night temperature is 20°C. or more.

9. Partial natural vernalization is induced in mustard Type 27 when the embryo develops under low temperature.

10. According to the prevalent categories, mustard Type 27 is neither a short-day nor a long-day plant, since it flowers under photoperiods of 10 hours as well as of 16 hours. But it is not indifferent to photoperiod.

11. Under all temperature ranges of the Almora climate, with an increase of photoperiod from 10 hours to 13 hours for the initial three weeks, plants from both untreated and vernalized seeds will flower significantly earlier. Photoperiods longer than 13 hours (up to 16 hours) induce similar effects in regard to the flowering date of mustard Type 27, and therefore 13 hours may be taken as the optimum photoperiod.

12. Under similar photoperiods greater shortening of the vegetative period is observed with increased temperature-range. Increased vegetative period during winter is primarily due to low temperature and not to short days. The optimum temperature for the second phase of development is 30°C.

13. Within limits, the effect of low temperature during the first phase, and of photoperiod during the second phase, in shortening the vegetative period of mustard Type 27 is of a quantitative nature.

14. Embryo of mustard subjected to low temperature, or seedlings subjected to optimum photoperiod, can produce similar shortening of vegetative period.

15. In the light of the experimental data presented, the original concepts of Lysenko's theory of phasic development of annual seed crops is not applicable to mustard Type 27, either in regard to the obligatory qualitative nature of changes produced by low temperature during the first phase, or the strict dependence of each phase on the completion of the preceding phase.

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ENTOMOLOGICAL INVESTIGATIONS ON THE LEAF-CURL DISEASE OF TOBACCO IN NORTHERN INDIA

V. BIOLOGY AND POPULATION OF THE WHITE-FLY VECTOR [*BEMISIA TABACI* (GEN.)] IN RELATION TO THE INCIDENCE OF THE DISEASE

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(With Plate III and five text-figures)

THE white-fly, *Bemisia tabaci** is a well-known pest of cotton in several parts of India and has been reported to be responsible for the periodic failure of some American varieties of this crop in the Punjab (Husain, 1933). The white-fly, which occurs in very large numbers, damages cotton by de-sapping the leaves, which consequently get disfigured and discoloured. It is also found on tobacco almost all over India, though it is not so common in well-known tobacco-growing areas, e.g. Guntur in the Madras Presidency, in south India. This white-fly is also reported to transmit leaf-curl of cotton in the Gezira district of the Sudan [Kirkpatrick, 1931] and leaf-curl of tobacco in Southern Rhodesia [Storey, 1932]. Thung [1932] reported that this species occurs in the Vorstenland districts of Java, where it is a vector of 'Kroepoek' disease of tobacco.

In India, *Bemisia tabaci* is not known to cause any virus disease to cotton, though its incidence on this crop is generally very high. In the case of tobacco, however, we have already conclusively shown that it is a very important vector of leaf-curl disease, which is common in North and Central India, [Pruthi and Samuel, 1937, 1939]. This white-fly has a large number of other food-plants in north Bihar (Pusa), a number of which also suffer from leaf-curl diseases, and in the case of some of them the white-fly has been shown by us to act as a vector of the disease [Pruthi and Samuel, 1941].

In view of the great importance of *Bemisia tabaci* as a vector of tobacco leaf-curl virus or viruses, the writers have studied its life and seasonal histories, range of food-plants, incidence on tobacco at different times of the year, etc., during the past four or five years at Pusa, and the results of these investigations are reported in the following pages :

* Silvestri (Entomologia Applicata, *Gli Insetti*, I, p. 401, 1934), considers *Bemisia gossypiperda* M. and L. to be a synonym of *B. tabaci* (Gennad.) (Gennadius, *Agric. ellenica*, 1889).

FOOD-PLANTS

The occurrence of *Bemisia tabaci* on several food-plants in the Punjab has been recorded by Husain and Trehan [1933, 1936]. In a previous communication, the present writers [Pruthi and Samuel, 1939] reported several cultivated and wild plants as hosts of this white-fly at Pusa. During the past two years, we have made a thorough survey of the alternate hosts of this species in the environs of Pusa and some other localities in north Bihar, and a list of all the plant hosts so far observed is given in Appendix I. Several of these plants show symptoms of some leaf-curl disease almost similar to that in tobacco and in the case of some we have, as already stated, experimental evidence that the virus is the same which causes leaf-curl in tobacco.

LIFE-HISTORY

Some observations on the life-history of *B. tabaci* made in cotton fields in India have been recorded by Misra and Lamba [1929], Husain and Trehan [1933], etc. Our observations differ in several important respects from those of the workers named above and are briefly described below :—

In Bihar, tobacco is usually sown in August and transplanted towards the end of September or early in October. The white-fly begins to make its appearance on this crop about the middle of October. It is generally found on the under surface of leaves, but all the leaves of a plant or all the plants are not equally infested ; in fact some are entirely free, but those leaves which are infested are generally fully covered with various stages of the fly. Therefore, in addition to causing the leaf-curl disease, the white-fly directly damages the leaves by de-sapping them and injecting their saliva therein. The honey-dew secreted by numerous nymphs is conducive to the development of sooty moulds on the leaves.

Copulation

The most active period of breeding is early autumn (September-October) and spring (February-March). Copulation occurs 2-6 days after emergence. An infested leaf, when closely examined, reveals a number of white-fly adults, sitting in groups of two or three in close contact with one another, and almost simultaneously shaking their wings preparatory to copulation. One male and one female are thus often seen together, but sometimes there are two males one on each side of a single female. The male as a rule dies within 24 hours after copulation, but the female after this process moves about restlessly for some time on the leaf surface, apparently in search of a suitable place for oviposition. By the time it actually deposits eggs, it acquires a full coating of powdery meal on its wings. The period between copulation and oviposition varies from one to two days in April-May and two to four days in October-December.

Oviposition

As the time for oviposition draws near, the female starts spinning on the under surface of the leaf, minute, irregular or circular patches made up of

network of thin, white fibres, composed of mealy-powder derived from its wings, which it scrapes off with its antennae and legs. The ovipositing female, therefore, generally looks somewhat discoloured, owing to the absence of disarrangement of its powdery stuff. After spinning for about 20 minutes, it deposits the first egg and covers it with a few powdery strands, and then lays another egg in close contact with the previous one and similarly covers it. Sometimes eggs are also laid almost entirely exposed. While laying eggs, the female raises the glandular hairs present on the surface to an upright position and clothes them densely with powdery meal. These hairs probably afford protection to the eggs against enemies.

Oviposition records were taken every month during the three tobacco seasons of 1936-39 (Table I). For this purpose, freshly emerged white-flies were taken and each pair was put in a micro-cage described by us in previous communications [1939, 1941]. The cage was fixed on the lower surface of a leaf of a young potted plant enclosed in a glass chimney. After twenty-four hours, the tube containing the pair was removed and adjusted on a fresh spot on the same leaf. This was repeated every day and eggs deposited in various spots were recorded.

The maximum number of eggs laid by a single female in captivity was 7 in 1936 (October), 69 in 1937 (September), 168 in 1938 (April) and 206 in 1939 (March). The maximum oviposition period varied from 9 to 12 days. The average number of eggs laid by a single female was 44 in 1937 and 77 in 1938. The female was found capable of laying up to a maximum of 56 eggs in twenty-four hours, and the egg-laying was distributed throughout the period. The highest number of eggs deposited by a female on cotton plants, according to Husain and Trehan [1933] was 119 in 18 days, the average being 28 in 1929 and 43 in 1930.

The meteorological conditions of the period during which the above observations were taken are summarized in Appendix II. An examination of the Appendix and Table I (containing the oviposition records) will show that the capacity for egg-laying is largely governed by the prevailing temperature and humidity conditions. As the temperature goes up, as is the case from March to May, the number of eggs laid per day increases, but the aggregate number remains almost the same. Under the opposite conditions (in December and January), the number of eggs deposited is considerably reduced and the oviposition period is prolonged.

Duration of immature stages

Husain and Trehan [1933] described the various stages of the white-fly, but their illustrations are not satisfactory. Therefore, drawings of the immature stages are included in this paper and the durations of various instars are briefly described below:—

The egg (Plate III, figs. 1-2).—The incubation period of the egg was 3-4 days in April-July, 3-10 days in August-March, the longest period observed being 7-10 days in December. The incubation period on cotton recorded in the Punjab was 3-33 days. The eggs are often preyed upon by a mite abundant on tobacco during August to October. They are also affected by intense cold, with the result that their hatching is indefinitely delayed.

TABLE I

Records of oviposition of Bemisia tabaci during 1936-39

Date of emergence	Date of beginning of oviposition	Total number of eggs laid and the number of days during which they were laid
1936—		
30 August	1 September	51 (4)
23 September	25 September	62 (4)
17 October	23 October	77 (4)
21 November	6 December	30 (6)
1937—		
7 January	14 January	36 (7)
17 February	21 February	47 (5)
25 March	30 March	55 (5)
20 April	23 April	39 (4)
17 May	19 May	58 (4)
14 June	18 June	35 (4)
9 July	12 July	31 (3)
6 August	8 August	27 (3)
1 September	4 September	69 (5)
2 October	4 October	50 (6)
2 November	5 November	42 (8)
6 December	11 December	35 (9)
1938—		
16 January	20 January	39 (12)
21 February	26 February	44 (5)
23 March	30 March	58 (5)

TABLE I—*contd.*

Date of emergence	Date of beginning of oviposition	Total number of eggs laid and the number of days during which they were laid
1938— <i>contd.</i>		
24 April	26 April	168 (5)
5 May	7 May	163 (5)
17 May	19 May	149 (5)
25 May	29 May	140 (5)
12 June	15 June	39 (4)
19 June	22 June	42 (3)
6 July	8 July	48 (4)
12 July	16 July	32 (3)
6 August	8 August	48 (4)
24 August	26 August	55 (4)
10 September	14 September	120 (7)
3 October	8 October	79 (6)
4 November	10 November	41 (5)
10 December	15 December	37 (9)
1939—		
25 January	30 January	33 (10)
20 March	23 March	206 (5)
16 April	20 April	131 (4)

Meteorological data for the above periods are given in Appendix II.

First instar nymphs (Plate III, fig. 3).—The young nymph casts its first moult in 3-5 days in August-September, 8-10 days in December-January and 3-5 days in May-June. The average duration of the first instar was 6 days. A portion of the cast skin is often seen adhering to the caudal end of the nymph but within a few minutes it dries up and gets blown off by wind.

Second instar nymph (Plate III, fig. 4).—The nymph moults in 2-6 days in August-September, 6-9 days in December-January and 1-4 days in April-May.

Third instar nymph.—The nymph moults in 2-7 days in August-September, 6-9 days in December-January and 2-4 days in April-May.

Pupa (Plate III, fig. 5).—The pupal period occupies 2-5 days in August-November, 4-6 days in December and 3-6 days in January. A freshly-formed pupa is generally thin and flat, sub-elliptical, light-yellow, but soon becomes convex and yellow, with the margin broadly crenulate.

The legs which are well developed in the first instar begin to degenerate in the succeeding instars, and are replaced by stump-like suckers in the second and third instars, while they are curved and unsegmented in the pupa. The dorsal spines, on the other hand, which are practically absent in the first instar gradually make their appearance in the subsequent instars, i.e. 3 pairs in the second and third instars, and seven pairs in the pupa. The number and arrangement of the dorsal spines of the pupa bred on cotton described by Husain and Trehan [1933] is also constant in the pupae bred from tobacco. But so far, we have not met with any case of the total absence of the dorsal spines mentioned by those authors.

The average duration of each of the three nymphal instars mentioned above is 4-5 days. The total period of the three instars was 12-21 days in August-March, 10-14 days in April-July and 18-24 days in October-December.

Duration of the life-cycle.—The life-cycle of the white-fly as observed by Husain and Trehan [1933] was 14-21 days during April to September, the longest being 107 days. According to our observations the life-cycle lasted 17-32 days during August-March in 1937-38 and 16-39 days in 1938-39. The longest life-cycle noticed was 39 days in December and the shortest 11 days in April.

In the laboratory, the white-fly completed twelve broods in the course of one year.

Emergence of adults.—The newly emerged insect often remains in contact with its empty pupal case for about 25 minutes, by which time the wings dry and unfold themselves to assume their normal shape and size. Freshly emerged adults have at first semi-transparent wings which in a day or two get covered with white mealy powder. Although emergence of adults generally takes place during the day, sometimes it also takes place at night.

Frequent collections of adults made from large rearing cages at different times of the year, have shown that the proportion of males to females is high during March-August, while it is low during September-February. The number of females emerging from the pupae is remarkably larger than that of males during winter months, viz. November-January.

The adults.—The abdomen of the male is creamy-yellow and tapering posteriorly; in the female, it is slightly bigger and broader and is distinctly yellow (Plate III, fig. 6). Both male and female prefer cool and shady places for breeding purposes. In summer, they hide in the day to avoid strong sun-light, and are active in the mornings and evenings. In winter, they are observed in the field between 8 A.M. and 12 noon and 2-30 and 5-30 P.M. While flying from one field to another they often exhibit whirling movements in the air, but when moving from one plant to another they invariably jump

Bemisia tabaci (Gen.)

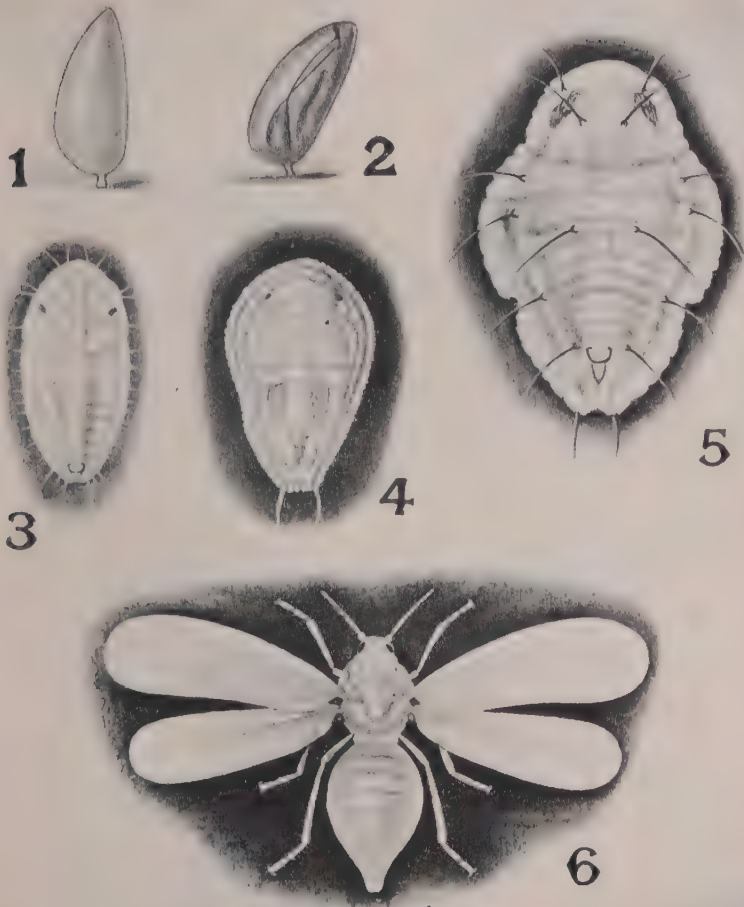


FIG. 1. A freshly laid egg ($\times 135$)

FIG. 2. The egg-shell after hatching of the young nymph ($\times 135$)

Note the longitudinal slit along one side

FIG. 3. Nymph, first instar ($\times 135$)

FIG. 4. Nymph, second instar ($\times 90$)

FIG. 5. Pupa ($\times 80$) Note the presence of eight pairs of spines (seven dorsal and one anal) and their arrangement.

FIG. 6. Adult white-fly, female ($\times 45$)

sometimes a jump may be as long as 20 feet or even more. Tobacco plants not in pots at a height of 30 feet from the ground level, became infested with white-flies, showing that they are capable of flying up to that height.

Longevity of the two sexes.—The duration of life was found to vary with the sexes. Males were usually short-lived, their average life in April-August being 4 days, and that of females 8 days. The average life in winter (November-January) for the two sexes was 7 and 12 days respectively. Copulation was also observed to influence the duration of life. In the case of males, the average duration in March-May before copulation was 4.5 days, and in November-January 6.5 days, while the corresponding figures for the two seasons after copulation were 3.8 and 5.5 days respectively. In the case of females, the average duration of life in March-May before copulation was 6 days, and in November-January was 8.5 days; the corresponding figures after copulation were 7.5 and 12.5 days respectively. These observations show that the length of life in males is shortened as a result of copulation, while in females it is increased.

When starved, the two sexes lived on an average for 1.5 and 2.5 days during March-May and November-January respectively.

Sexual dimorphism

There is sexual dimorphism among the adults and pupae of the white-fly and the two sexes can be recognized easily. The adult female differs from the male by having a comparatively stouter abdomen and longer wings, and its pupa is bigger in size than that of the male.

Parthenogenesis

The phenomenon of parthenogenesis mentioned by Husain and Trehan [1933] has also been observed by us. At Pusa it was noted both in the spring and autumn. Freshly emerged females were kept isolated in micro-cages, allowing them no chances for sexual reproduction. One to two weeks later, 12-37 eggs were laid per female, and the progeny arising from them consisted of only males, which were smaller in size than those produced normally.

THE POPULATION OF THE WHITE-FLY ON TOBACCO AND SOME OTHER HOSTS AT DIFFERENT TIMES OF THE SEASON

We have already stated that there is a large number of wild and cultivated plants which act as hosts for the white-fly. Sunn-hemp, which is one of its important food-plants, is sown at Pusa about the middle of May. The white-fly appears on it in June, when the plants are about two weeks old. On *urid*, *patwa* and *arhar* it appears early in August having migrated to them from *duranta* and several other weeds, e.g. *Solanum nigrum*, *Vernonia cinerea*, *Euphorbia hirta*, *Ageratum conyzoides*, *Anisomeles ovata*, *Launea asplenifolia*, *Scoparia dulcis*, etc. The white-fly multiplies rapidly on the above mentioned plants up to September, then there is a gradual decline in its numbers and about the middle of November there is practically no white-fly on these plants. Sunn-hemp remains in the field up to the end of September for manurial purposes and up to the end of January for seed purposes. *Patwa* lasts up to the end of January, but *arhar* remains till March.

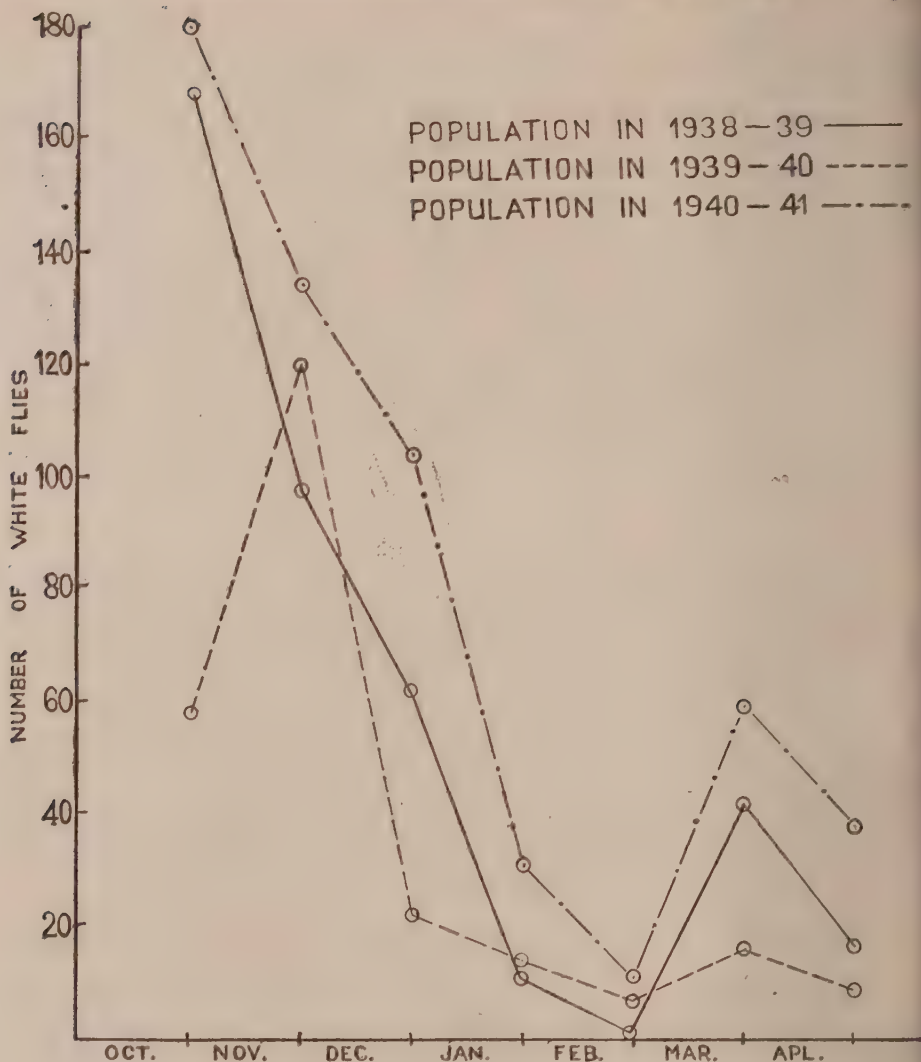


FIG. 1. The population of white-fly during 1938-41*

When tobacco seedlings are transplanted in the field about the end of September, the white-flies, start migrating to them from duranta, sunn-hemp, *Ageratum*, *Launea*, and almost all the above named weeds. The migration of adults was observed in the fields from 7-30 to 10-30 A.M. and 2-30 to 5-30 P.M. in October-November. In the first two weeks of October, eggs were found on majority of the seedlings, while a large number of gravid females were still in the process of ovipositing. Hatching of the eggs took place in the field about the third week of October, and adults of the first brood appeared about the middle of November. The development of the

* The number of white-flies given on the vertical axis are those found in a randomized area of three-quarters of an acre of tobacco plants.

white-fly is rather slow during December and January. Thus four to five generations only are completed on tobacco crop up to the end of March. As the vector has been observed to complete 12 generations in a year in the laboratory, it is obvious that the remaining generations are completed on its other hosts during March to the end of August. Thus the white-fly is able to thrive almost throughout the year on account of its having a wide host range. It overwinters in the form of pupae, of which some get actually killed by frost, fungus (*Alternaria* sp.), and parasites.

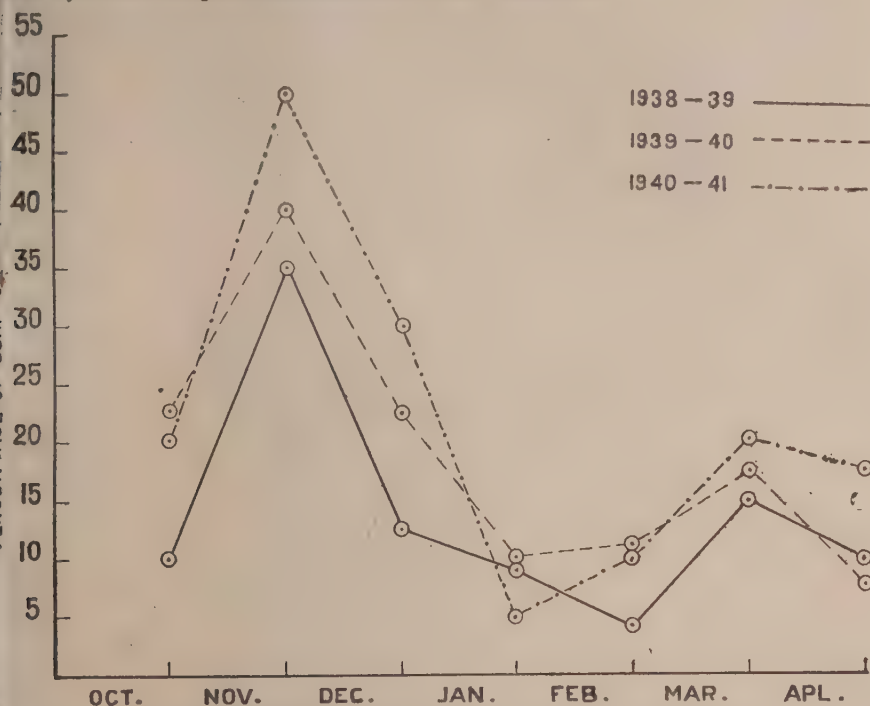


FIG. 2. The incidence of leaf-curl disease during 1938-41

Sticky boards were set up during August-December on the bunds of some fields, in which sunn-hemp, *urid*, soya-bean, *meth*, tobacco, etc. were growing, to note the extent and time of flights of the white-flies. The data collected showed that maximum number of captures were secured in September-October thus coinciding with the planting time of tobacco.

In 1938, a three-quarter acre tobacco plot (I P Hybrid 142) sown and transplanted respectively in September and October was selected for the purpose of estimating the changes in the population of the white-fly at different times of the year. The plot consisted of 44 rows with 110 plants in each row, and of these, to avoid border effect, 36 rows with 100 plants in each row were selected for taking the observations, the total number of plants under examination being 3,600. The plants were randomized, and about 72 plants distributed in 36 rows at the rate of two plants in each row were examined weekly. In addition to this, daily counts were also taken on 10 plants which had also been selected at random. The weekly population of

the white-fly was recorded by carefully examining all the leaves of the entire plants with a hand lens, and counting the number of different stages (nymph, pupae and adults) of the vector present on each plant. Weekly counts of the leaf-curl plants were also recorded along with the population of the white-fly. Observations were taken on these lines throughout the tobacco season, beginning from the third week of October up to the middle of April. Similar observations were also taken during 1939-40 and 1940-41 in a field in which August-sown tobacco was transplanted in September or early in October (normal time). The data of incidence of the white-fly and leaf-curl collected during the three years are graphically shown in Figs. 1 and 2. From the data it is evident that the number of various stages as well as the adults decreased from the first week of November up to the second week of January, after which there was again an increase which continued up to the second week of April when the crop was harvested. The graphs in Figs. 3-5 show the meteorological data separately for the three years (1938-41) during which the incidence of the white-fly and leaf-curl disease was recorded.

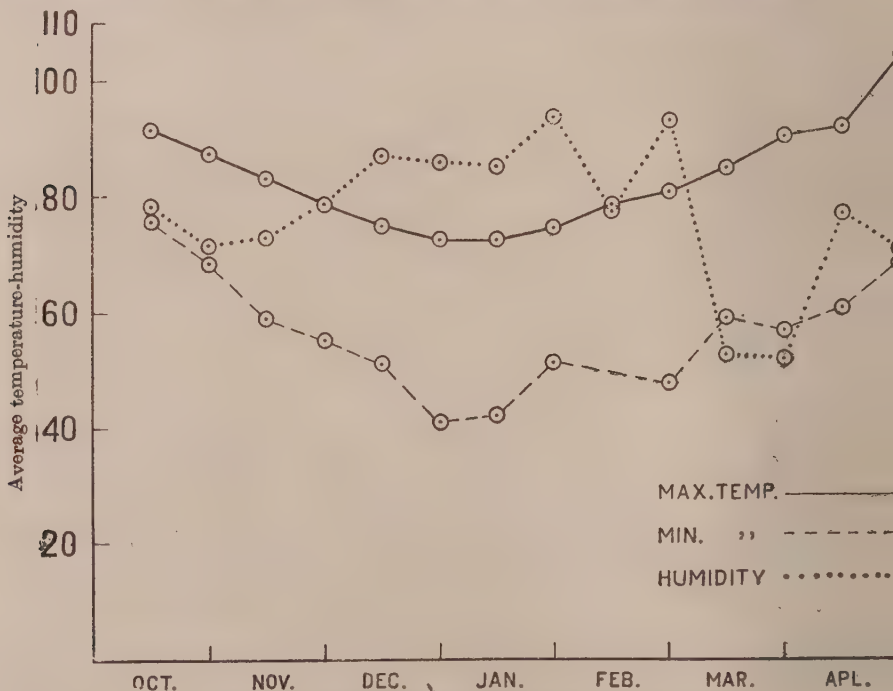


FIG. 1. Meteorological data* during 1938-39

CORRELATION BETWEEN THE INCIDENCE OF WHITE-FLY AND TOBACCO LEAF-CURL

In order to ascertain whether any correlation existed between the incidence of the white-fly and the intensity of leaf-curl disease on tobacco crop

* The temperatures are given in degrees F.

Seedlings were raised every month from July to December during 1939-40, and the seedlings from each nursery were successively transplanted in three-quarter acre plots. Weekly census of the white-fly (all stages) together with the incidence of diseased plants occurring in each plot every week were recorded. The data thus obtained are given in Table II.

In the case of July-August lot, the white-fly appeared in the fourth week of September and increased rapidly in numbers during October and November, but decreased from December onwards. The leaf-curl disease was first shown by eight plants in the last week of September, followed by a slight rise in October and November, but there was a considerable fall in December and January. Thus the intensity of leaf-curl corresponds with the rise and fall in the population of the white-fly. It may be pointed out that the incidence of leaf-curl in the fourth week of September shows that infection had already taken place in the nursery stage.

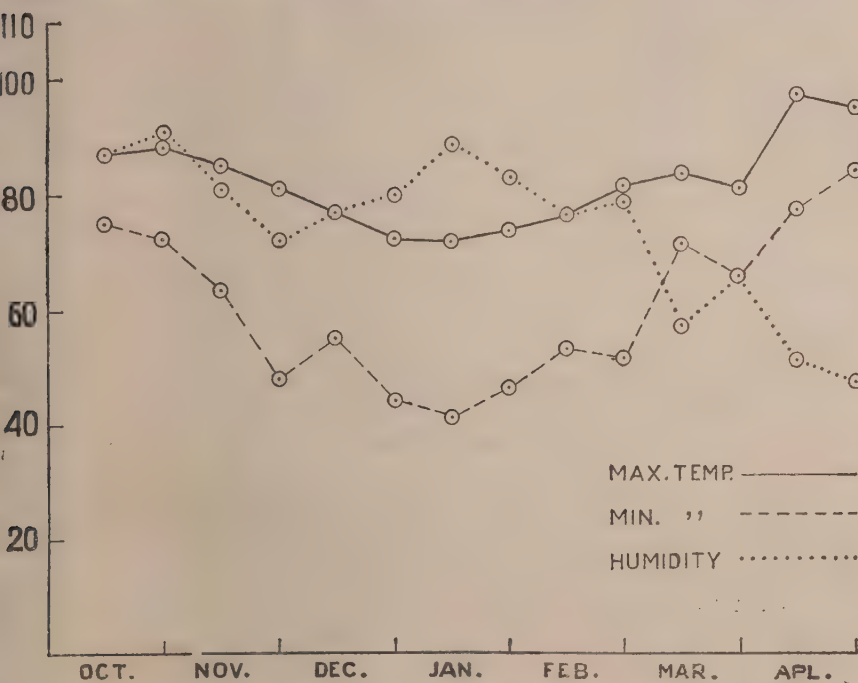


FIG. 4. Meteorological data* during 1939-40

In the August-September lot, the white-fly appeared in large numbers in the second week of October. The incidence at the end of November was almost the same as in the July-August lot. It, however, decreased from December onwards. Diseased plants, which were first noticed in the third week of October decreased in number in November and December, and no further incidence of disease was observed in the rest of the season.

* The temperatures are given in degrees F.

In the September-October lot, the white-fly appeared in the fourth week of October, but enormously increased in November, followed by a gradual decrease in the rest of the season. The corresponding incidence of leaf-curl, which first appeared in November and December was practically negligible.

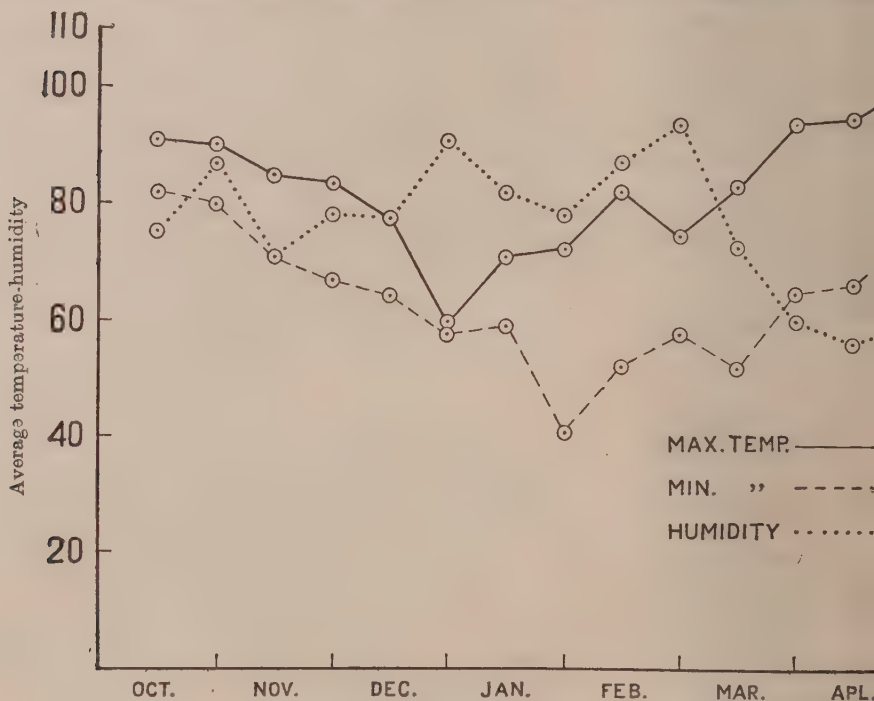


FIG. 5. Meteorological data* during 1940-41

In the October-November lot, the white-fly began to appear in small numbers in the fourth week of December, and its incidence remained very low in the following months. Only two plants showed disease in the third week of December.

In the November-December lot, the low incidence of white-fly which was observed in the fourth week of January, remained almost steady up to March. The corresponding incidence of leaf-curl was also noticed to be very low.

In the December-January lot, white-fly began to appear in the fourth week of March and no increase was noticed in its numbers in April. The disease did not appear in the plot at all.

The foregoing observations show that the incidence of leaf-curl disease is closely dependent upon the population of the white-fly. Furthermore, it can be concluded that infection of seedlings in the nursery stage also takes place if they are kept exposed for a considerable time before being transplanted.

* The temperatures are given in degrees F.

the crop year 1939-40

No.	Sowing Transplanting	March			Monthly total	April				Monthly total	GRAND TOTAL	Remarks
		2	3	4		1	2	3	4			
1	July-August—											
	White-flies	4	4	296	
	Leaf-curl plants	25	
2	August-September—											
	White-flies . .	4	3	7	16	5	4	9	246	
	Leaf-curl plants	23	
3	September-October—											
	White-flies . .	2	6	6	14	4	3	7	104	
	Leaf-curl plants	2	
4	October-November—											
	White-flies . .	1	2	2	6	35	
	Leaf-curl plants	2	

Total number of
plants under
observation in
each lot was
154.

Data for November and

bacco (I P H-142) during the crop year 1940-41

ary		Monthly total	February				Monthly total	March				Monthly total	April				Monthly total	GRAND TOTAL	Remarks
3	4		1	2	3	4		1	2	3	4		1	2	3	4			
1	...	1	4	1	1	1	7	2	6	1	...	9	48	Total number of plants under observation in each expo- sed and pro- tected lot was 154.
...	1	2	3	1	5	2	...	8	6	7	1	...	14	43	
...	1	...	1	2	1	3	2	4	10	8	6	4	...	18	135	
...	1	...	1	1	1	1	1	4	5	5	2	...	12	109	
1	1	5	1	1	2	3	3	7	2	15	2	5	1	...	8	98	
1	1	4	1	2	4	2	9	4	2	6	61	
...	...	2	3	4	6	13	3	4	4	...	11	94	
...	...	4	2	2	1	3	8	6	2	1	...	9	62	
...	8	1	1	10	12	18	9	...	39	73	
...	4	2	5	1	12	14	19	22	...	55	73	
...	2	1	4	2	9	10	6	13	...	29	59	
...	6	1	2	5	14	8	11	5	...	24	52	
4	2	7	1	1	7	1	10	7	9	18	...	34	54	
1	2	4	1	2	...	3	3	1	2	...	6	18	
1	1	3	2	1	1	1	5	1	1	1	...	3	11	
...	1	1	1	1	1	3	1	...	1	...	2	6	

The above observations were continued during the year 1940-41, when, like the previous year, nursery seedlings were raised under two sets of conditions, viz. one set was exposed to nature, while the other was protected under an insect-proof cover up to the time of transplanting. The data thus collected are given in Table III.

From a close examination of the data it is evident that as in the case of the previous two years, the incidence of the disease was dependent on the population of the white-fly, and further seedlings under insect-proof covers in the nursery showed lower incidence of the white-fly and correspondingly lower incidence of the disease than those which were exposed.

NATURAL ENEMIES

An unidentified Chalcid parasite of the pupa of *Bemisia tabaci* on cotton has been recorded by Husain and Trehan [1933]. At Pusa three new Chalcid parasites, viz. *Prospaltella smithi* Silv., *Pteropteryx bemisiae* Mani (to be described elsewhere) and *Eretmocerus masii* Silv., were noticed parasitizing the pupa of this white-fly while infesting several food-plants, viz. tobacco, sun-hemp, *Ageratum*, soya-bean, duranta, cowpea, gingelly, chillies, zinnia, cotton, etc. The parasites were noticed during the months of November-January, and of the three species, *P. bemisiae* appeared to be most common.

Parasitized pupae could be easily distinguished from the normal ones by the dark colour of their entire body except the vasiiform end which was reddish-brown. The pupal cases of the parasitized white-fly left after the emergence of the parasites and white-flies can also be easily distinguished. They have a tiny circular hole in the region of the thorax on the dorsum in addition to dark or reddish-brown colouration which very often persists even after the emergence of the parasites.

In order to estimate the degree of parasitization of the host on these various plants, the leaves from each plant were collected at random and the parasitized and non-parasitized pupae were counted on each leaf. The parasitism was found to vary from 1.5 to 5.1 per cent. On cotton, where intense breeding of the white-fly in the rearing cages was noticed, parasitism was found to be as high as 62.73.5 per cent.

SUMMARY

1. The biology of the white-fly, *Bemisia tabaci*, the vector of the leaf-curl virus disease of tobacco, was studied in tobacco fields at Pusa for five years.
2. The white-fly has a large number of alternate food-plants, some of which also suffer from virus diseases and have been proved to be the alternate hosts of the tobacco leaf-curl virus. A complete list of the food-plants with the time of the year when the white-fly is found on them and its intensity is given in Appendix I.
3. The population of the white-fly on tobacco crop studied at different times of the year showed that it is highest in autumn (up to the middle of November), goes down in winter and rises again in March.
4. The incidence of the disease in tobacco is dependent on the population of the white-fly.
5. A brief account of the natural enemies of the white-fly is given.

ACKNOWLEDGEMENTS

Our sincere thanks are due to the Imperial Economic Botanist for allotting a tobacco plot for our observations, besides many other facilities provided for our work at the Botanical Sub-station, Pusa. The Imperial Mycologist has kindly identified the entomogenous fungus found infesting the pupae of the white-fly.

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APPENDIX I

Food-plants of the white-fly, *Bemisia tabaci* Gen., in the environs of Pusa (Bihar) and remarks on the incidence of leaf-curl disease in them

Food-plant	Time of occurrence of the white-fly and its degree of infestation	Time of occurrence of leaf-curl and its degree of incidence
SOLANACEÆ		
<i>Psidium annuum</i> *	Oct.-Dec.; Feb.-March; severe in Feb.-March.	Oct.-Dec.; 15-25 per cent
<i>Stramonium</i> *	Feb.-March; moderate	Sept.-Oct.; 75 per cent
<i>Nicotiana glauca</i>	Oct.-Feb.; severe
<i>Nicotiana glauca</i>	Feb.-March; moderate	Aug.-March; 6-15 per cent
<i>Nicotiana glauca</i>	Oct.-Dec.; moderate	Nov.-Dec.; 1 per cent
<i>Nicotiana glauca</i>	Nov.-Dec.; moderate	Oct.-Dec.; 2 per cent
<i>Nicotiana glauca</i>	Oct.-Nov.; moderate
<i>Nicotiana glauca</i>	Ditto
<i>Nicotiana glauca</i>
<i>Nicotiana glauca</i>	Oct.-Nov.; moderate	Severe in Oct.-Nov.; 25-30 per cent
<i>Nicotiana glauca</i>	Aug.-Dec.; severe in Nov.	Aug.-Dec.; 30-40 per cent
<i>Nicotiana glauca</i>	Oct.-Nov.; moderate
<i>Nicotiana glauca</i>	Nov.-Jan.; moderate	Dec.; trace
<i>Nicotiana glauca</i>	Oct.-Dec.; severe	Oct.-Dec.; 40 per cent
<i>Nicotiana glauca</i>	Oct.; moderate	Oct.-Nov.; 1 per cent
<i>Nicotiana glauca</i>	Oct.-Nov.; Feb.-March; moderate.	July; March; 5 per cent
<i>Nicotiana glauca</i>	Oct.-Dec.; Feb. moderate	Nov.-Jan.; 2-5 per cent
<i>Nicotiana glauca</i>	Aug.-Oct.; moderate
LEGUMINOSÆ		
<i>Trigonotis hypogaea</i> *	June-Aug.; moderate	July-Aug.; 5-12 per cent
<i>Trigonotis cajan</i> *	Dec.-Feb.; Aug.-Oct.; very low	Dec.; trace
<i>Trigonotis arietinum</i> *	Dec.-Jan.; very low	Dec.-Jan.; 5-10 per cent
<i>Trigonotis ternatea</i>	March-April; very low

* Plants which show symptoms of leaf-curl disease almost similar to that in tobacco.

** The virus on these plants is the same which causes leaf-curl in tobacco.

APPENDIX I—*contd.*

Food-plant	Time of occurrence of the white-fly and its degree of infestation	Time of occurrence of leaf-curl and its degree of incidence
LEGUMINOSEÆ— <i>contd.</i>		
<i>Crotalaria juncea</i> **	June-Oct.; severe	Aug.-Nov.; 10-15 per cent
<i>Dolichos lablab</i>	Oct.-Dec.; very low
<i>Ervum lens</i>	Dec.-Jan.; very low
<i>Glycine hispida</i> *	Oct.-Nov.; moderate	Oct.-Nov.; trace
<i>Medicago sativa</i>	March-April; very low
<i>Melilotus parviflora</i>	Sept.-Oct; very low
<i>Phaseolus calcaratus</i> *	Oct.-Nov.; March-April; moderate	Oct.-Nov.; trace
<i>Phaseolus mungo</i> *	March-April; severe	March-April; 70-80 cent
<i>Phaseolus radiatus</i> *	July-Oct.; moderate	Aug.-Sept.; 30-40 per cent
<i>Phaseolus vulgaris</i>	Dec.-Jan.; very low
<i>Pisum arvense</i>	Ditto
<i>Pisum sativum</i>	Ditto
<i>Trifolium alexandrinum</i>	March-April; low
<i>Vigna catjang</i>	Oct.-Dec.; very low
COMPOSITÆ		
<i>Ageratum conyzoides</i> **	July-Nov.; moderate	July-Dec.; 20-25 per cent
<i>Calendula officinalis</i> *	Dec.-Jan.; very low	Dec.-Jan.; 1-2 per cent
<i>Carthamus tinctorius</i> *	Dec.-Mar.; fairly severe	Dec.-Feb.; 10 per cent
<i>Cosmos bipinnatus</i> *	Dec.-Jan.; very low	Dec.-Jan.; 1-2 per cent
<i>Inula (Vicoa) vestita</i> *	Aug.-Sept.; Nov.-Dec.; very low	Dec.-Mar.; 30-40 per cent
<i>Launea asplenifolia</i> **	Jan.-Mar.; June-Sept.; severe in Feb.-March	July-Mar.; 50 per cent
<i>Vernonia anthelmentica</i> *	Sept.-Dec.; moderate	Sept.-Nov.; 5-10 per cent
<i>Vernonia cinerea</i> **	July-Oct.; very low	July-Feb.; 5-10 per cent
<i>Xanthium strumarium</i>	Jan.-Mar.; moderate
<i>Zinnia elegans</i> **	Aug.-Oct.; moderate	Aug.-Jan.; 15-20 per cent

* Plants which show symptoms of leaf-curl disease almost similar to that in tobacco.

** The virus on these plants is the same which causes leaf-curl in tobacco.

APPENDIX I—*contd.*

Food-plant	Time of occurrence of the white-fly and its degree of infestation	Time of occurrence of leaf-curl and its degree of incidence
MALVACEÆ		
<i>Althaea rosea</i> *	Aug.-Dec.; Jan.-April; moderate	Aug.-Dec.; 5-15 per cent
<i>Asiosappium herbaceum</i>	Ditto
<i>Abiscus cannabinus</i> *	July-Nov.; moderate	Aug.-Dec.; 10-15 per cent
<i>Abiscus esculentus</i> *	Aug.-Oct.; moderate	Aug.-Dec.; 5-10 per cent
<i>Abiscus rosa-sinensis</i> *	Aug.-Oct.; Feb.-March; moderate	All round the year; 40-50 per cent
<i>Ada cordifolia</i>	Aug.-Oct.; low
<i>Ada rhombifolia</i> **	Aug.-Oct.; moderate	July-Feb.; 5-15 per cent
LINACEÆ		
<i>Linum usitatissimum</i>	Sept.-Nov.; low
CRUCIFERÆ		
<i>Rassica campestris</i>	Nov.-Jan.; very low
<i>Rassica napus</i> *	Ditto	Nov.-Feb.; 10-15 per cent
<i>Rassica oleracea</i> *	Ditto	Ditto
<i>Rassica oleracea</i> var. <i>botrytis</i> *	Ditto	Ditto
<i>Rassica juncea</i>	Ditto
<i>Rassica rapa</i> *	Ditto	Nov.-Feb.; 2-5 per cent
<i>Raphanus sativus</i> *	Ditto	Nov.-Feb.; 5-10 per cent
CUCURBITACEÆ		
<i>Cucumis melo</i>	April-May; low
<i>Cucumis sativus</i> *	Aug.-Oct.; low	Sept.-Nov.; 1 per cent
<i>Cucurbitaria vulgaris</i> *	July-Sept.; low	Oct.-Nov.; 1 per cent
<i>Cucurbita acutangula</i> *	Ditto	Sept.-Oct.; trace
<i>Cucurbita aegyptiaca</i> *	Ditto	Ditto
<i>Trichosanthes anguina</i> *	Ditto	Ditto
<i>Trichosanthes dioica</i>	Sept.-Nov.; March-April; moderate

* Plants which show symptoms of leaf-curl disease almost similar to that in tobacco.

** The virus on these plants is the same which causes leaf-curl in tobacco.

APPENDIX I—*contd.*

Food-plant	Time of occurrence of the white-fly and its degree of infestation	Time of occurrence of leaf-curl and its degree of incidence
UMBELLIFERÆ		
<i>Coriandrum sativum</i> . . .	Jan.-Feb.; low
LABIATÆ		
<i>Anisomeles ovata</i> * . . .	Aug.-Oct.; moderate . . .	Feb.; Sept.-Nov.; trace
<i>Nepeta ruderalis</i> . . .	Aug.-Nov.; moderate . . .	Feb.;
<i>Ocimum basilicum</i> . . .	Aug.-Oct.; low
<i>Ocimum sanctum</i> . . .	Ditto
EUPHORBIACEÆ		
<i>Euphorbia hirta</i> ** . . .	July-Sept.; March-April; low . . .	July-Dec.; 5-10 per cent
<i>Euphorbia heterophylla</i> . . .	Aug.-Oct.; moderate . . .	March-April;
<i>Euphorbia hypericifolia</i> . . .	Ditto
<i>Euphorbia prostrata</i> . . .	Ditto
CONVOLVULACEÆ		
<i>Convolvulus arvensis</i> . . .	Oct.-Dec.; very low
<i>Coccinia indica</i> . . .	Ditto
<i>Impomœa batatas</i> * . . .	Oct.-Dec.; moderate . . .	Nov., trace
<i>Impomœa reptens</i> . . .	Ditto
AMARANTACEÆ		
<i>Achyranthes aspera</i> * . . .	Aug.-Sept.; moderate . . .	Nov.-Dec.; Aug.-Dec.; 5-12 per cent
<i>Amarantus gangeticus</i> . . .	Sept.-Nov.; very low
<i>Amarantus spinosus</i> . . .	Ditto
<i>Amarantus viridis</i> . . .	Ditto
<i>Celosia cristata</i> . . .	Ditto

* Plants which show symptoms of leaf-curl disease almost similar to that in tobacco.

** The virus on these plants is the same which causes leaf-curl in tobacco.

APPENDIX I—concl'd.

Food-plant	Time of occurrence of white-fly and its degree of infestation	Time of occurrence of leaf-curl and its degree of incidence
CAPPARIDACEÆ		
<i>Leome chelidonii</i> . . .	Sept.-Oct.; very low
<i>Leome viscosa</i> . . .	Ditto
ACANTHACEÆ		
<i>Quellia prostrata</i> . . .	July-Sept.; low
<i>Cesamum indicum</i> * . . .	Sept.-Nov.; moderate .	Sept.-Oct.; 8-15 per cent
VERBENACEÆ		
<i>Perodendron infortunatum</i> * .	Aug.-Oct.; very low .	Sept.-Dec.; 10-25 per cent
<i>Duranta plumieri</i> * . . .	Oct.-Jan.; March-April ; moderate	Oct.-Jan.; 5-20 per cent
TILIACEÆ		
<i>Perchorus capsularis</i> . . .	Oct.-Dec.; moderate
<i>Perchorus acutangulus</i> . . .	Ditto
URTICACEÆ		
<i>Cannabis sativa</i> * . . .	Feb.-April ; moderate .	Feb.-March; 1-2 per cent
CHENOPODIACEÆ		
<i>Chenopodium album</i> . . .	Oct.-Dec.; very low
SCROPHULARINEÆ		
<i>Scoparia dulcis</i> ** . . .	July-Oct.; moderate .	July-Dec.; 10-15 per cent
ROSACEÆ		
<i>Rosa centifolia</i> . . .	Feb.-April ; very low
GERANIACEÆ		
<i>Oxalis corniculata</i> . . .	Sept.-Nov.; very low
GRAMINACEÆ		
<i>Euphismenus burmanni</i> . . .	Sept.-Dec.; fairly high
COMMELNIACEÆ		
<i>Commelina benghalensis</i> . .	Sept.-Dec.; fairly high

* Plants which show symptoms of leaf-curl disease almost similar to that in tobacco.

** The virus on these plants is the same which causes leaf-curl in tobacco.

APPENDIX II

Meteorological data for the periods during which oviposition records were taken

Year	Average temperatures during the period (°F.)		Relative humidity (per cent)	Remarks
	Maximum	Minimum		
1936—				
30 August—4 September	88·8	80·2	91·0	
23—29 September	89·8	77·2	87·0	
17—27 October	84·0	70·4	82·0	
21 November—12 December	78·5	49·0	82·0	
1937—				
7—21 January	74·7	42·5	72·0	Temperature records not taken
17—26 February	68·3	55·5	94·0	
25 March—4 April	93·5	66·5	51·0	
20—27 April	101·5	70·5	49·0	
17—23 May	103·7	74·5	61·0	
14—22 June	
9—15 July	
6—11 August	91·0	79·0	83·0	
1—9 September	92·0	80·0	80·0	
2—10 October	83·0	70·0	89·0	
2—13 November	82·8	61·5	78·0	
6—20 December	70·5	43·0	83·0	
1938—				
16 January—1 February	74·0	47·5	84·0	
21 February—3 March	74·9	46·0	74·0	
28 March—4 April	96·0	60·5	40·0	
24 April—1 May	97·0	73·5	48·0	

APPENDIX II—*concl.*

Year	Average temperatures during the period (°F.)		Relative humidity (per cent)	Remarks
	Maximum	Minimum		
38— <i>contd.</i>				
5—12 May	93.0	70.5	84.0	
17—23 May	95.0	78.5	72.5	
25 May—4 June	90.0	78.0	87.0	
12—19 June	93.5	77.0	93.0	
19—25 June	93.0	79.3	78.0	
3—12 July	91.5	80.5	85.0	
12—19 July	88.5	78.2	91.0	
3—12 August	87.5	78.2	93.0	
24—30 August	91.5	81.0	80.0	
10—21 September	93.5	79.2	89.0	
2—14 October	92.4	76.7	79.0	
4—15 November	84.0	59.7	76.0	
10—24 December	74.5	47.2	94.0	
39—				
5 January—9 February	75.5	53.0	94.0	
20—28 March	93.0	59.5	49.0	
6—24 April	95.0	62.5	46.0	

BIOLOGICAL CONTROL OF THE COTTON STEM WEEVIL, *PEMPHERULUS AFFINIS* FST., IN SOUTH INDIA

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(With Plate IV and ten text-figures)

PEMPHERULUS AFFINIS Fst. commonly known as the cotton stem weevil and one of the major pests of both exotic and indigenous varieties of cotton in south India, is widely distributed in the cotton-growing districts of Madras. With a view to furthering the possibilities of its control the Indian Central Cotton Committee sanctioned a small scheme to study its distribution in India, both on cotton and its alternative hosts, in conjunction with a search for parasites and predators. The scheme was put into operation in October 1935 and continued for a period of three years. The present paper records the results obtained in this preliminary investigation.

METHODS

At the outset the need for exact information on the incidence, habits and reactions of the stem weevil was felt. Continuous and quantitative field studies were made to trace the annual course of weevil-breeding, with particular reference to the time and character of its incidence in cotton. Biological surveys of the important cotton-growing tracts and quantitative collection and examinations of alternate host plants were also undertaken. In the course of these studies over 55,000 cotton plants, 23,000 alternate host plants and 14,000 specimens of parasites have been handled.

The mass of data accumulated during the period may be conveniently dealt with under three main heads: (1) studies on the weevil, (2) studies on parasites and predators, and (3) a concluding part summarizing the present position of our knowledge of the problem.

PREVIOUS KNOWLEDGE

The information so far available may be briefly summarized as follows. Ramakrishna Ayyar [1918] and Ballard [1922] published general outlines of the life-history and the different stages of the insect. It was then known to occur only in Coimbatore, Salem, Trichinopoly, Madura, Ramnad and Malabar districts. The life-cycle is recorded to range from 77 to 99 days: 6-8 days as egg, 35-57 days as larva and 9-10 days as pupa. The life-span of the adult is recorded to be about 36 days. The average egg-laying capacity is about 15 eggs per female with a maximum of 30. The emergence period extends for about a month. Among its alternate host plants are included a dozen species such as *Althaea rosea*, *Hibiscus esculentus*, *H. cannabinus*, *Corchorus olitorius*.



- A. A typical *Pempherulus*-infested Cambodia cotton plant.
- B. Cotton stems depicting variations in number, nature, position, shape and size of gall formation.
- C. Cotton stems with bark peeled off showing early course of attack and tunnelling round the stems.
- D. Longitudinal section through infested cotton stems revealing the damage caused inside the stems by the grubs.

la spinosa, *Triumfetta* spp., *Ficus religiosa*, *Hibiscus rosa-sinensis*, *Calotropis*, *Antia*, *Melia azadirachta*, *Abutilon indicum* and *Dombeya*. No natural enemies, either parasites or predators, were known. Indigenous varieties of cotton were supposed to be less susceptible to weevil attack.

I. STUDIES ON THE WEEVIL

DISTRIBUTION

Madras Province

As these studies were based on brief reconnaissance tours in certain selected cotton-growing tracts in the province the data cannot be regarded as either exhaustive or conclusive. Broadly speaking, the distribution of the pest is restricted to the southern parts of the Madras province, where it has developed to a major pest of cotton. In the northern districts the weevil may be considered to be almost absent as a pest of cotton, its place being, in a way, taken by the Buprestid borer, *Sphenoptera gossypii*, though far less destructive. To more precise, *Pempherulus* is now definitely known to occur either in cotton allied food plants in the whole of Coimbatore district, almost the whole of Madras district, the southern portion of Tinnevely, the whole of Malabar and the southern border of south Canara and portions of the adjoining native states of Cochin and Travancore. In Malabar and south Canara the insect has been noted only on allied food plants but not cotton. Stray specimens of the weevil have also been received from a few of the northern districts like Madras. It is practically absent in the Ceded Districts.

Other parts of India

A brief visit to Bihar, United Provinces, Gujerat and Hyderabad (Deccan) forms the basis for these observations. The weevil is not known as a serious pest of cotton in any of these provinces in India. It has, however, been noted to breed in alternate host plants like *Hibiscus esculentus* at Pusa and Sasamusa, Surat and Dehra Dun. From Dehra Dun it has also been found on other allied plants such as *Urena lobata*, *Althaea rosea*, *Sida rhombifolia* and *S. acuta*. The weevil has not so far been observed in cotton or alternate food plants from Hyderabad.

Distribution outside India

The species is reported to infest cotton in Burma and Philippines. It has been ascertained that the weevil is absent in Indo-China, Federated Malay States, Australia, New Zealand, Brazil, British West Indies, South Africa, Rhodesia, Uganda, Sudan, Egypt, U. S. S. R. and Ceylon.

The genus *Pempherulus* and its distribution*

The genus *Pempherulus* comprises only five known species whose distribution is confined to the Indo-Malayan zone as may be seen from the data furnished.

P. affinis Fst.—India, Burma and Philippines

P. habena Pascoe.—Singapore, Malacca and Philippines

* The writer is indebted to the Imperial Institute of Entomology, London, and U. S. Bureau of Entomology, Washington, for this information.

P. pleurostigma Fst.—South India

P. picta Heller.—Tenasserim (Lower Burma)

P. trilineata Pascoe.—Batchian (Dutch East Indies)

Three of these species are restricted to Indo-Burman region. The genus be regarded as Indian or Indo-Burman in origin.

SEASONAL HISTORY

Trend of incidence in seasonal crops

A systematic collection of cotton plants and detailed examination of same week after week and month after month were arranged. The data recorded in a definite and uniform plan so as to afford an idea of the progress of incidence, seasonal history, number of generations, density of population, course of parasitism and other controlling factors. The material collected cannot be considered thoroughly representative, owing to limitations of area and staff, and also to the inevitable factor of the erratic distribution of the pest. Notwithstanding these handicaps, the quantitative data secured during the period indicated the main trends.

TABLE I
Percentage infestation in seasonal crop

Month	1935-36		1936-37		1937-38	
	No. of plants examined	Percentage of infestation	No. of plants examined	Percentage of infestation	No. of plants examined	Percentage of infestation
September . . .	1,200	0.0
October . . .	3,158	3.2	224	1.3	1,470	12.8
November . . .	803	23.2	832	4.7	2,302	14.8
December . . .	1,381	28.4	539	68.0	727	35.4
January . . .	1,228	55.0	684	83.0	2,292	54.0
February . . .	1,577	77.2	2,278	89.9	1,464	57.0
March . . .	869	91.7	345	90.1	668	59.0
April . . .	498	88.0	321	93.5
May . . .	455	99.0	109	98.2
June . . .	154	100.0	79	98.7
July . . .	260	100.0
August . . .	210	100.0

An analysis of the data recorded (Table I) for the three years reveals a general uniformity in the trend of infestation from October to March. It reveals that weevil incidence grows in intensity as the season advances. The seasonal history is roughly characterized by the occurrence of three generations though considerably overlapping from October to March. The first of these generations roughly covers the period from October to December. The second is not very clearly defined but occupies a period up to middle of February.

During this period the overlapping of broods is so heavy that generations are almost indistinguishable. A third brood may commence in February and extend far into April. Beyond this period, owing to heavy overlapping, broods are absolutely indistinguishable. Despite this overlapping, two more supplementary broods, though feeble, could be indistinctly traced, if the crop is retained till the end of August.

Though these general remarks are applicable to the three seasons under review, there have been considerable variations in the nature and extent of infestation and progress during different seasons. In the early stages of the crop, say within three to four weeks, there is a total absence of infestation as may be seen from 0 per cent in September. The fresh initial wave of incidence is visible in October and the progress of this brood is distinct. Oviposition probably commences during the month and probably to a slight extent by the latter part of the previous month and stray, newly hatched grubs form the only noticeable stages during the period. The percentage of infestation ranged from 1.3 to 12.85. Small incipient galls are apparent even at this stage.

Early part of November reveals the presence of medium-sized grubs, a proportion of which reaches maturity towards the close of the month. The percentage shows an increase and ranges from 4.7 to 23.2. All stages including prepupae, pupae and adults along with emergence apertures and large well-formed galls are available in December when the percentages vary from 28.4 to 68. The first generation is now almost nearing completion and the partial wave of emergence may be noticed. By January the second generation may be said to have commenced with the attack ranging from 54 to 83 per cent. In spite of three successive broods in the season, the population does not show a steady increase throughout. Towards the middle of February the live population reaches its peak, varying from 45.4 to 98.5 stages per 100 plants (1937 and 1938). Thereafter, the population shows a gradual decline, ranging from 15.7 to 28.7 (Table II).

TABLE II

Trend of live population

(Live population per 100 plants)

Month	1936-37	1937-38
October	1.3	13.1
November	4.1	10.7
December	72.9	31.8
January	68.2	42.7
February	98.5	45.4
March	28.7	15.7

Possible factors.—The actual causes of the fall in population are, however, not definitely known. On the other hand it is obvious that only successful waves of adult emergence at every generation can maintain or augment population density.

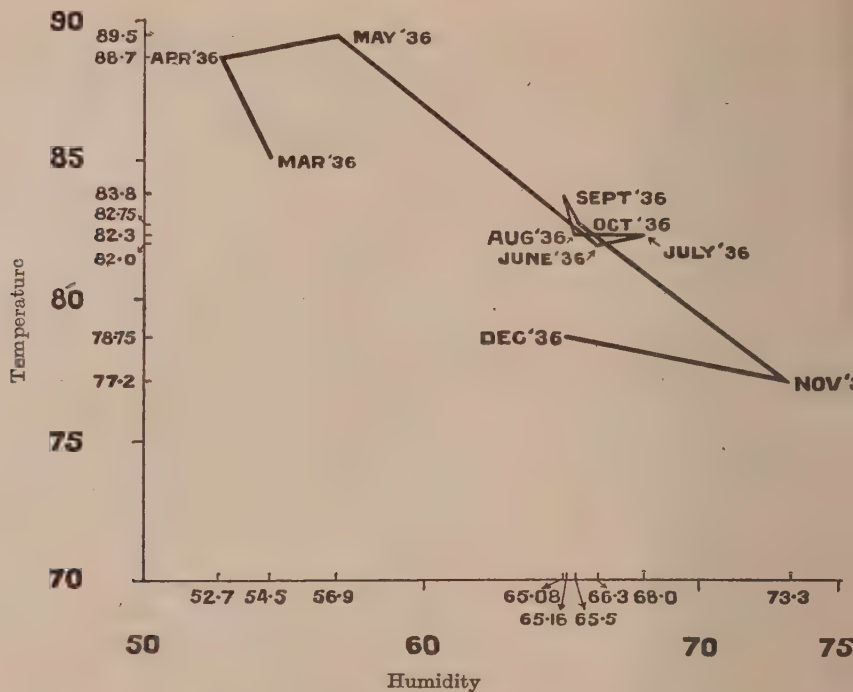


FIG. 1. Temperature-humidity curve, 1936

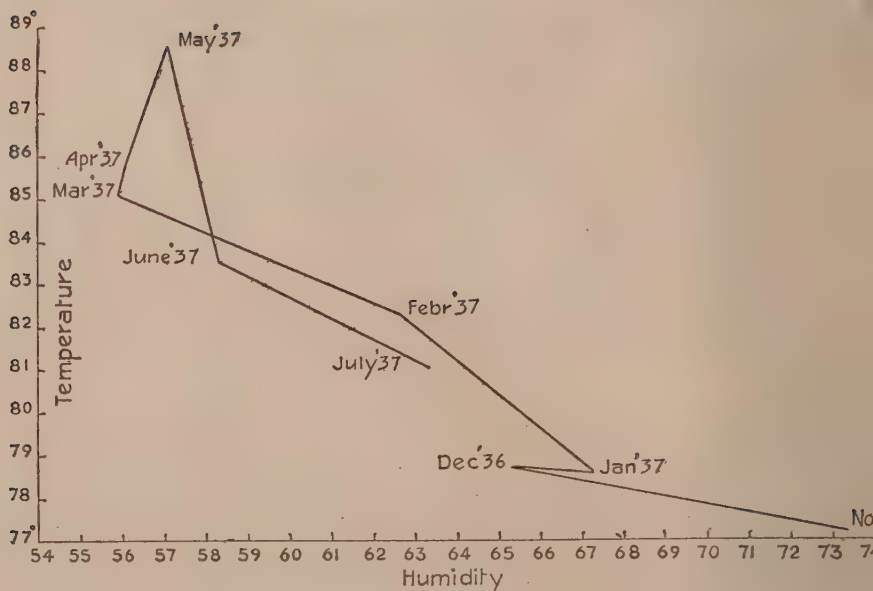


FIG. 2. Temperature-humidity curve, November 1936 to July 1937

At the prevailing ecological conditions at this stage seem to operate against the weevil. Among these, the increased resistance inherent in the plants by pronounced gumming which showed a marked increase during the productive phase of the plant, may be counted as one. Another probable factor in operation may be the unsuitability of the crop due to changes in plant constitution. This has in a way been corroborated by chemical analysis. Usually, the prevailing dry spell due to higher temperatures and lower humidities (Figs. 1, 2 and 3) and the slight increase in the parasitic element (Tables II and IX) may also have had their share in contributing to this decline.

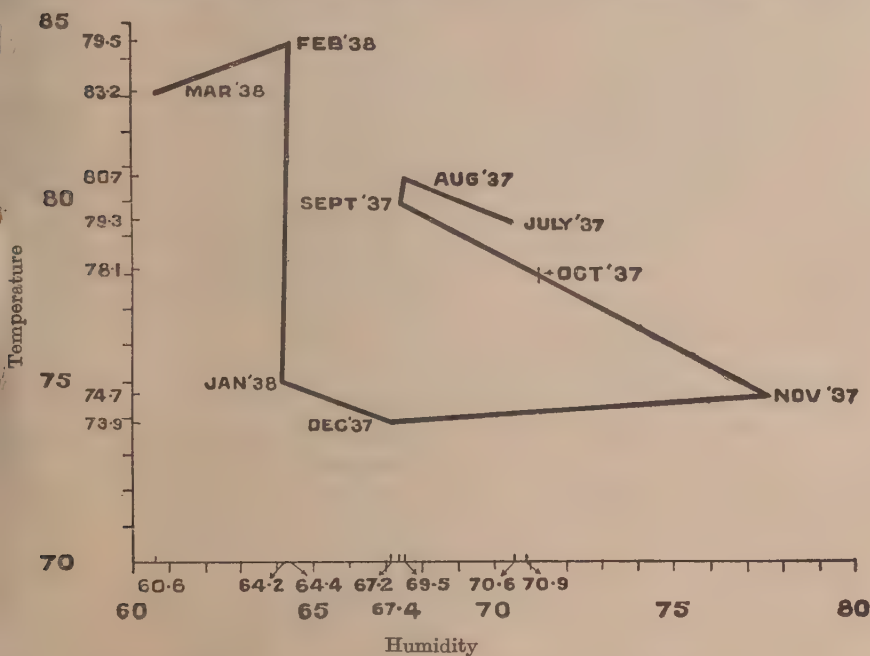


FIG. 3. Temperature-humidity curve, 1937-38 (average mean)

End of incidence in off-seasonal crops

The decrease in the density of population in the seasonal crop by about February raised the question of the influence of the season marked by high temperatures and low humidities as compared with the age of the crop. With a view to shedding some light on this problem, an off-seasonal crop was raised for the first time in February 1937 and again in 1938. From Table III it may be noted that there was a phenomenally heavy incidence and parasitism in 1937, which served to suggest the importance of the age of the crop as against physical factors. The data for 1938 have not been significant for the purpose. It may, therefore, be seen that no satisfactory answer can be given unless the experiment is repeated for a series of years and correlated with meteorological data.

TABLE III
Percentage infestation in off-seasonal crops

Month	1937		1938	
	No. of plants examined	Percentage of infestation	No. of plants examined	Percentage of infestation
March	640	5.6
April	300	36.7	676	10.0
May	253	79.0	570	22.0
June	324	100.0	1,268	29.0
July	632	100.0	2,154	47.0
August	1,355	100.0	1,588	87.0
September	no crop		767	93.0

Status of the weevil as pest of cotton

A systematic collection and examination of all dead plants from the field afford striking proof of the status and destructiveness of the weevil. From the data furnished (Table IV) nearly 97 per cent of the total mortality in cotton is caused by *Pempherulus*.

TABLE IV
Mortality in plants

Month	Number of plants	Percentage
November 1935	1,087	95.0
December 1935	590	94.0
January 1936	380	98.0
February 1936	657	100.0
March 1936	325	98.0
April 1936	205	100.0
May 1936	179	100.0
June 1936	60	100.0
July 1936	31	100.0
November 1936	700	98.0
December 1936	300	100.0
January 1937	200	100.0

Influence of temperature and humidity on the weevil

The irregular distribution of the insect in different years and the remarkable variation in incidence in different localities necessitated an extensive study of its reactions under different combinations of temperature and humidity. Apparently its heavy incidence in certain localities indicated that there are definite optimum localities having special environmental conditions, particularly temperature and humidity. Therefore, a study of its physical ecology was undertaken. The temperature was controlled by using different capsules in an electric incubator. Humidities were adjusted by using sulphuric acid of different dilutions. The results obtained have formed the subject of a separate paper [Krishna Ayar 1941]. These are briefly summarized in the following paragraphs.

(i) Each phase of the insect's life has distinct and differential requirements of temperature and humidity for its survival and development.

(ii) The adults are unable to withstand high temperatures for considerable duration. Their upper thermal death-point is about 122° F. when they are unable to stand exposure for six hours. At 113° F. it takes 48 hours to kill them. Between 113° F. and 106° F. a very much longer period of exposure is necessary to produce lethal effect. Changes of humidity do not seem to affect them at these high temperatures.

(iii) For maximal functional activity and longevity a temperature range of 90°-98° F., associated with humidity 60-80 per cent, is the optimum. The duration of life of the adults may extend to three months under these conditions. Changes in humidity also exercise great influence on the longevity of the adults, 60-80 per cent being the most favourable (Table V). At 100° F. they live for shorter periods.

TABLE V

Influence of humidity on the longevity of the adults

Humidity (per cent)	Longevity of females (days)	
	Mated	Unmated
0	2.6	2.4
20	3.8	3.4
40	13.3	14.1
60	13.2	39.3
80	13.5	34.7
100	13.0	18.0

At 91° F. and 73 per cent humidity, a maximum of 91 days averaging 50.5 for 45 individuals has been recorded.

(iv) Reproduction is much reduced above 100°F., unless when the climate is very favourable. The egg-laying capacity decreases with rise in temperature. At 91°F. the average production per female is 46 eggs, at 92.9°F. 29.2, at 100°F. 25.3, at 106°F. 4.5 and at 113°F. 0.7 eggs, the last being a collapsed condition.

(v) A wide range of tolerance is exhibited at each temperature with regard to humidities for oviposition. At 100°F. the optimum is between 60 and 100 per cent relative humidity, at 93°F. between 60 and 80 per cent and at 91°F. is about 70-75 per cent.

(vi) The incubation period is not much affected by variations in humidities at normal temperatures.

(vii) The upper limit of viability of egg is a little below 100°F. At 100 per cent humidity there is partial hatching, at 80 per cent there is complete hatching, but the best level for hatching and survival is 100 per cent relative humidity.

(viii) Eggs and early stage grubs are very sensitive to desiccation and high mortality is caused by changes in this factor; medium and mature grubs require medium humidities, while prepupae and pupae withstand and develop even in lower humidities like 40—20 per cent and 0 per cent. A higher humidity of 100 per cent is not conducive to the development of older stages and leads to fungal attack.

(ix) Mating retards longevity of the adults. At 60 per cent humidity and 93°F. the unmated male can live to the maximum period of 98 days, whereas a mated one lives only 58 days. Similarly, an unmated female lives longer than three months, whereas its mated sister perishes after 54 days.

(x) At favourable temperatures and humidities there is no difference in the duration of life of the different sexes but, under unfavourable conditions the males succumb earlier.

(xi) The duration of life is much affected by food (Table VI).

TABLE VI

Duration of life at 93°F. and 80 per cent relative humidity

Mated or unmated		Sex	Maximum longevity without food (days)	Maximum longevity with food (days)
Unmated	Male	4.0	23.8
Do.	Female	4.4	34.7
Mated	Male	4.0	18.5
Do.	Female not allowed to oviposit	4.6	13.2
Do.	Female allowed to oviposit	6.0	18.6

The results described in Table VI explain to a great extent why *Pempherus* is more abundant under irrigated conditions and in more succulent varietal-like Cambodia.

Alternate food plants

An interesting phase of the investigation of the weevil is the study of its plants other than cotton. The earlier accounts, besides being vague and conflicting, fail to provide the exact species of plants in many cases. No data on incidence, locality, susceptibility and status are available for any species. It is, therefore, evident from the outset that the host plants were imperfectly known. These studies were restricted in scope, being confined mostly to localities in Coimbatore and its environs. A few occasional collections from adjoining forests were also made. These have not only brought to light a number of hitherto unrecorded food plants, but also provide valuable information on the exact species of plants, the nature and character of infestation, the changes in the habits of the insect in relation to plant species and a series of new parasitic habits peculiar to these changed habits. A concise summary of the data is given in Table VII.

TABLE VII

Name of the alternate host	Natural order	Total number examined	Average per cent of infestation	Highest per cent of infestation	Remarks
<i>Persea rhomboidea</i> *	Tiliaceae	4,999	69.6	100	Doubtful host
<i>Persea olitorius</i>	"	1,519	27.0	51.5	
<i>Persea trilocularis</i>	"	684	1.2	2.8	
<i>Persea acuta</i> *	Malvaceae	5,116	16.7	80.0	
<i>Persea spinosa</i>	"	160	20.7	73.7	
<i>Persea glutinosa</i> *	"	190	12.6	91.3	
<i>Persea rhomboidea</i> *	"	278	6.5	10.0	
<i>Persea rhombifolia</i> *	"	109	4.6	50.0	
<i>Persea astrum coromandelianum</i> *	"	4,607	14.9	41.5	
<i>Persea vitifolius</i> *	"	1,324	6.1	40.0	
<i>Persea ficulneus</i> *	"	740	3.0	16.6	Doubtful hosts since no live stages have been actually recovered
<i>Persea esculentus</i>	"	381	14.4	30.5	
<i>Persea cannabinus</i>	"	321	35.2	80.0	
<i>Persea sinuata</i> *	"	396	0.3	16.7	
<i>Persea surattensis</i>	"	304	1.3	20.0	
<i>Persea corchorifolia</i>	"	258	4.7	7.4	Doubtful hosts since no live stages have been actually recovered
<i>Persea hirtum</i>	"	356	10.4	52.7	
<i>Persea glaucum</i>	"	531	1.1	5.5	

Nine plants among these, marked with asterisk, were recorded for the first time as alternate hosts for *Pempherus affinis*. The discovery of such a large number of plants, most of them occurring wild in nature, has set the problem of control of the pest on a different footing. It has made it clear that

the mere observance of a close period between two cotton crops will not actively control the pest under the conditions prevailing at Coimbatore and a prolonged close period.

Amongst the several hosts, *Triumfetta rhomboidea* is the most favoured wild plants but adults emerging from this host do not oviposit freely on it. It is not clear whether this preference is due to race differences or food preferences. The importance of the discovery of unrecorded parasitic fauna on these food plants will be dealt with under parasites.

Reaction of Pempherulus with reference to food

Studies on insect dietetics are of great practical importance in affording clues for devising preventive and control measures. A series of experiments were therefore, conducted to determine the effect of different kinds of food on the fecundity and duration of life of the females of *Pempherulus* under identical physical conditions and the results obtained have formed the basis of the matter of a separate paper [Krishna Ayyar, 1940, 3].

Mere supply of water does not seem to have any beneficial effect on the life-duration or reproductive powers. An exclusive carbohydrate diet produced a remarkable increase in duration of life and also, to a limited extent, fecundity. Raisin, whose composition includes a small proportion of proteins and fats, besides carbohydrates, has yielded best results. It seems to constitute an ideal food among those tested in respect of all activities, inclusive of fecundity and longevity. From an average of about four eggs without any special food as high an average as 76.1 eggs per female with a record number of 164 eggs as maximum per female has been obtained on a raisin diet. The results of a few tests on oviposition responses in relation to oviposition sites, such as roots, flower buds, etc. are also presented.

Original home and habitat

The investigation of the original home and primitive environment of the pest may afford the key to its present status and eventual control by being the most promising source of efficient parasites [Myers, 1931]. Previous studies on the weevil gave no indications of the original home of the pest. The weevil came into prominence with the introduction of an exotic variety of Cambodia cotton from Cochin China some 30 years ago. On this assumption, as also because of its long association with this variety of cotton, it was supposed that the weevil was also imported from the same country. But a study of its geographic distribution coupled with that of its food plants and parasites largely renders this assumption open to question. It has already been established that the genus does not extend beyond the Indo-Malayan zone. It is, therefore, evident that the immediate ancestors of *Pempherulus affinis* and its relatives must have had their origin in this region. Further, it is a well-known fact that an insect in its native habitat is usually well controlled by its natural enemies. But *Pempherulus* has no effective natural enemy in cotton field and the pest recorded must be regarded as of recent association since none is mentioned in the previous literature. These and other considerations suggested that the pest might have commenced to infest cotton comparatively recently. The studies made in parts of Malabar district pointed that wild *bhindi* (*Hibiscus* species) should have had an older association with weevil than cotton, since the i

ferred the former, when both were grown together. Besides, it was found that *Hibiscus esculentus* in localities where cotton was completely absent. Further studies revealed that the insect is found infesting wild plants in hill tracts and forests in distant parts of India, such as Malabar in the south and Nagpur in the north. Its association with such wild plants as *Triumfetta rhomboidea*, *Sida acuta*, *S. rhomboidea*, *Urena lobata* in virgin forests far away from any cotton cultivation would appear to be still older and more primitive than its association with *Hibiscus esculentus*. Among these, *T. rhomboidea* appeared to be the most common in both heaviness of infestation and parasitism. With it is associated a parasitic fauna not met with in cotton tracts. These findings are highly suggestive of the possibility that *Pempherulus* is indigenous to India with some of these wild plants (possibly *T. rhomboidea*), serving as its original or primitive habitat. Further studies in this line would prove extremely useful and interesting.

II. STUDIES ON THE PARASITES

The investigations conducted in this line may be conveniently discussed under four main heads :—

- (a) Parasites in association with cotton
- (b) Parasites in association with food plants other than cotton
- (c) Parasites imported from other provinces
- (d) Mass-breeding experimental releases and recoveries

PARASITES IN ASSOCIATION WITH COTTON

One predatory mite and six species of Hymenopterous parasites were obtained from *Pempherulus* during the course of the present studies. These are listed below :—

Acarina—*Pediculoides ventricosus* Newpt. (Tarsonemidae)

Braconidae—*Spathius critolaus* Nixon

Chalcidoidea—

Euderus pempheriphila Ramkr and Mani

Eupelmus sp.

Aplastomorpha calandrae (How.)

Eupelmus urozonus Dalm.

Unidentified Braconid (*Microbracon* sp. ?) as also a Chalcid

Pediculoides ventricosus Newpt. (*Acarina*—*Tarsonemidae*): It is known to have a world-wide distribution. Among its hosts may be counted the eggs, pupae and even adults of a wide range of soft-bodied insects, particularly insects of stored products and others that live in partial or complete concealment. Being an external parasite it feeds by sucking out body fluids from soft integumented insects. The life-history of the mite has been worked out by many authors [Taylor, 1937] and is well known. It has a short life span; the eggs and young ones develop inside the mother and are given birth to as adults though at this stage their size is small. Soon after it commences feeding, it becomes globular in size with a small anterior projecting head. It attacks the immature stages of *Pempherulus* in the laboratory but has never been observed in nature in the field. Very often the grubs together with their parasites are devoured. Its utility as a means of pest control is extremely doubtful.

Extent of total parasitism

The course of parasitism was followed in relation to three seasonal sown in September and two off-seasonal crops between March and A (Fig. 4). The data are presented in Table VIII.

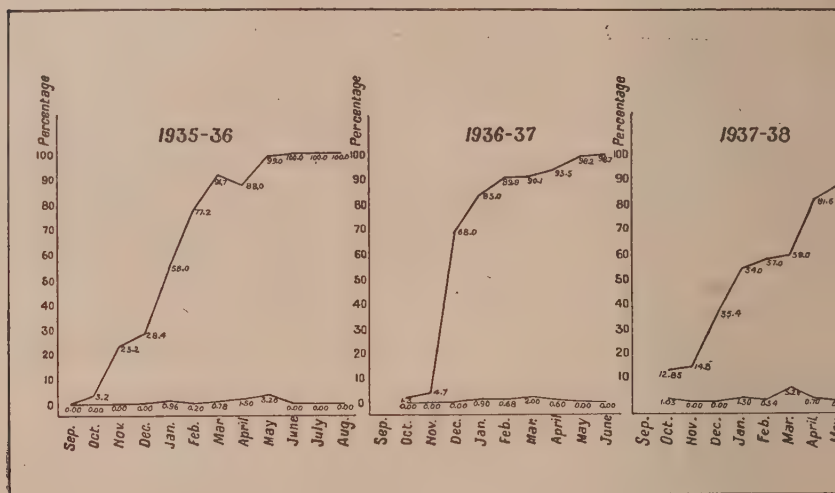


FIG. 4. Percentage infestation and parasitism in seasonal crop

TABLE VIII

Infestation and parasitism in seasonal crops

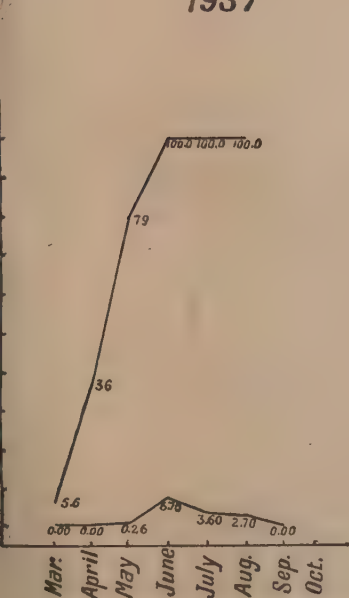
Month	1935-36		1936-37		1937-38	
	Percent- age of infesta- tion	Percent- age of para- sitism	Percent- age of infesta- tion	Percent- age of para- sitism	Percent- age of infesta- tion	Per- age pa sit
September	12.85	1.
October	3.2	..	1.3	..	14.80	..
November	23.2	..	4.7	..	35.40	..
December	28.2	..	68.0	..	54.00	..
January	55.0	1.90	83.0	0.90	57.00	0.
February	77.2	0.59	89.9	0.68	59.00	5.
March	91.8	4.70	90.1	2.00	59.00	5.
April	88.0	1.50	93.5	0.60
May	100.0	3.20
June	100.0
July	100.0

TABLE IX

Infestation and parasitism in off-seasonal crops

Month	1937		1938	
	Percent- age of infesta- tion	Percent- age of para- sitism	Percent- age of infesta- tion	Percent- age of para- sitism
March	5.6
April	36.7	..	10.9	..
May	79.0	0.26	22.1	0.70
June	100.0	6.70	29.3	0.50
July	100.0	3.60	47.0	1.40
August	100.0	2.70	87.8	0.75
September	93.7	3.20

1937



1938

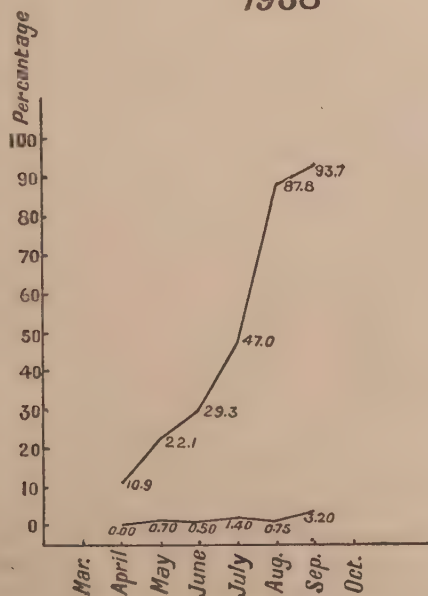


FIG. 5. Percentage incidence and parasitism in off-seasonal crop

From Table VIII it may be evident that the rate of parasitism during three seasons shows marked irregularity which does not admit of any explanation. The total parasitism did not exceed 5.2 per cent in the seasonal crop and 6.7 per cent in the off-seasonal crop. Parasitism was evident in the first brood itself in the seasonal crop for the year 1937 but the peak period would appear to be February-March when second and later broods overlapped. Off-seasonal crops show a greater regularity in parasite incidence (Fig. 5)

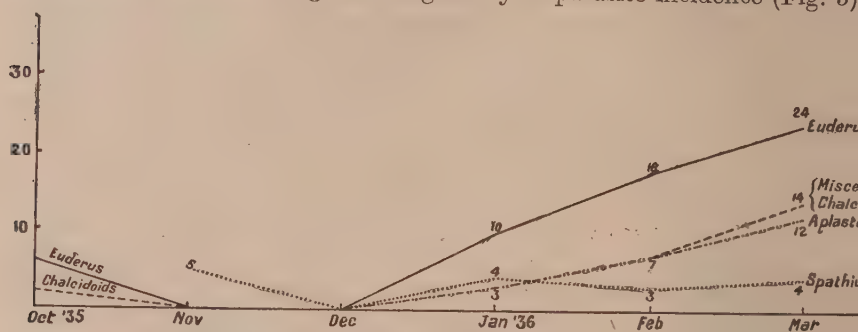


FIG. 6. Parasitism in seasonal crop, 1935-36

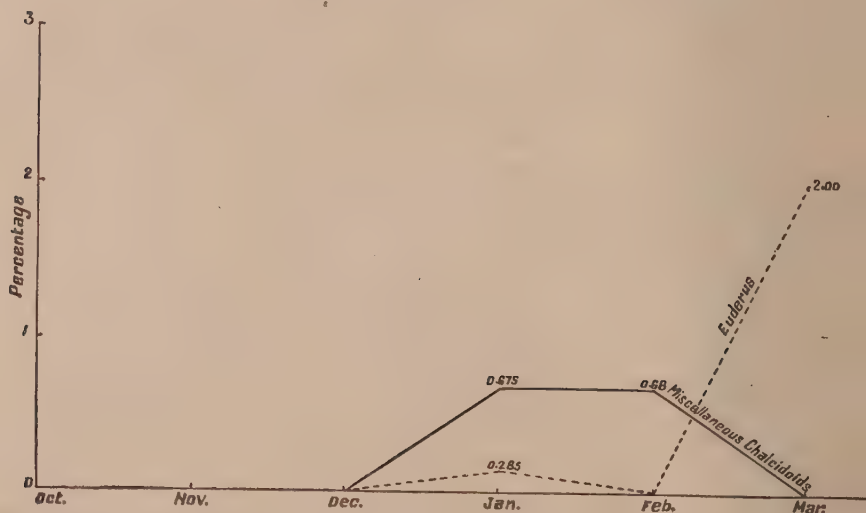


FIG. 7. Parasitism in seasonal crop, 1936-37

Seasonal incidence of parasitism

Among parasites, *Spathius critolaus* generally appears early in the season during the first generation of the pest. Its occurrence, though in small numbers should, however, be deemed important since it happens at a time when pest incidence is low. The importance of *Euderus pempheriphila* consists in its numerical superiority. It is comparatively abundant in January as a

June to August. *Aplastomorpha* and *Eupelmus* sp. occur in some numbers in the season—June to August—when the crop is to be removed. The other species were only of occasional occurrence and their role in the control of the pest may be considered to be insignificant (Figs. 6-10).

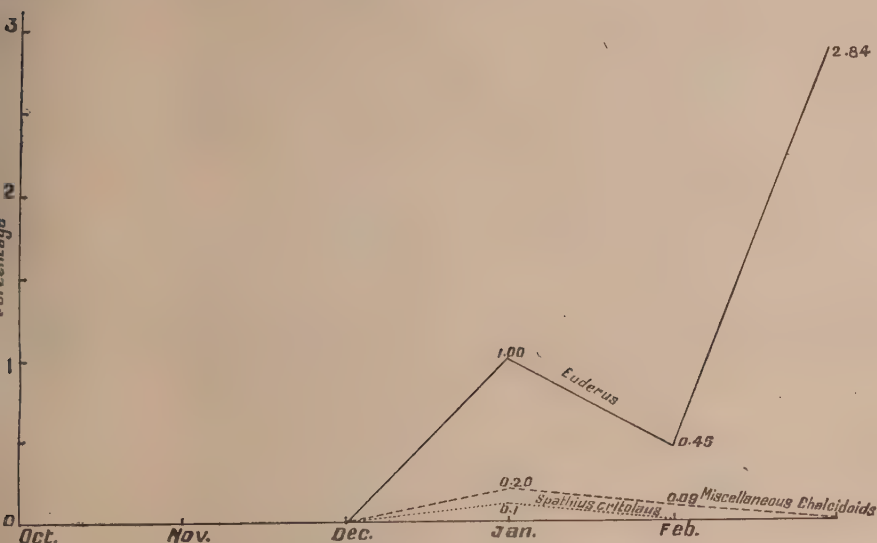


FIG. 8. Parasitism in seasonal crop, 1937-38

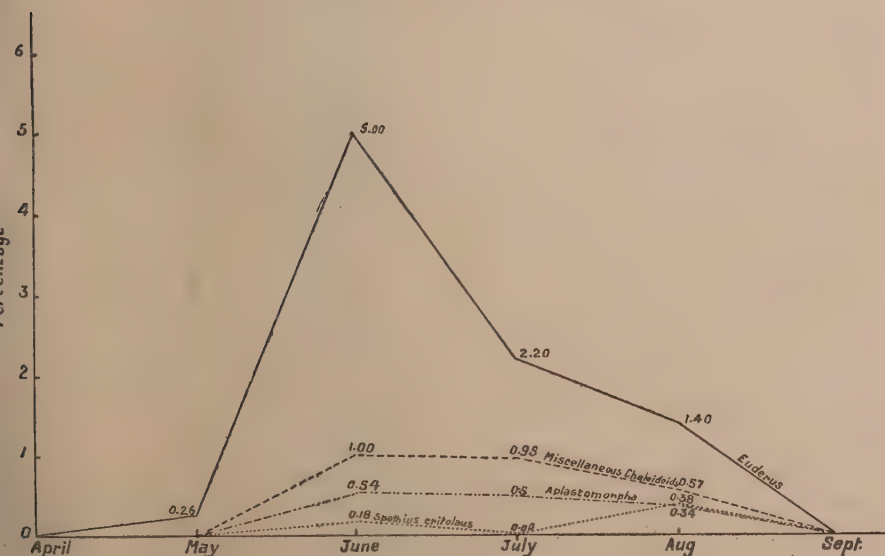


FIG. 9. Parasitism in off-seasonal crop, 1937

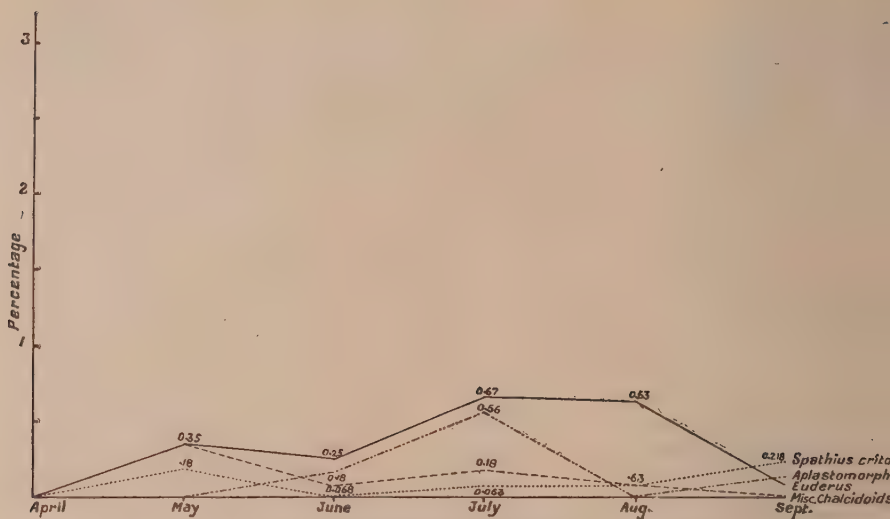


FIG. 10. Parasitism in off-seasonal crop, 1938

Biology of the parasites

The habits and life-history of a few have been studied in detail. A detailed study of others has not been possible.

Spathius critolaeus Nixon is a primary, ectophagous, larval parasite. A small proportion of the females is winged. Mating is not essential for oviposition. The females search out the grubs in the galleries, paralyse them by stinging and then oviposit. Only active, healthy and fairly mature grubs appear to be chosen as hosts. Generally one egg is laid on each host, although a female can lay up to a maximum of seven eggs per day, the average being two to three. The maximum number of eggs laid by a female was 53. The pre-oviposition period is normally two days. The egg stage lasts for one or two days. The larvae on hatching, feed externally on the fluid contents of the host for about four to five days. When full grown, they spin cocoons around the bodies. The prepupal and pupal periods are two or three days and eight days, respectively. The adults emerge by making a circular opening first in the cocoon and later in the bark of the stem. The males generally emerge earlier (by one to three days) than the females. Parthenogenetic progeny are all males. The entire life-cycle may vary from 14 to 25 days, according to the season. The duration of life of the adult varies considerably with the nature and availability of food. When fed on the nectar of cotton flowers, they were found to live up to 5½ months. No case of hyper-parasitism has been noticed so far. A detailed account of this parasite has already been published [Krishna Ayyar, 1940, 1].

The parasite has two alternate hosts : (1) the amaranthus weevil *Hypolixus truncatulus* (Curculionidae) and (2) the cotton stem-borer Bostrychid, *Sinoxylon sudanicum*. The discovery of these has been useful for rearing the parasite under laboratory conditions when *Pempherulus* is not available in the field. An account of their mass-breeding is furnished in a later paragraph.

Euderus pempheriphila Ramkr. and Mani is a dark, small-sized, Eulophid with a tiny ovipositor, attacking medium-sized grubs, found near the bark, and yet it is the most numerous amongst the weevil parasites collected from cotton fields. The female stings the host completely paralyses it and lays in most cases a single egg, loosely and indiscriminately on any part of the host larva. Even when more than one egg is laid, it is only one that develops to maturity. The larva on hatching feeds voraciously on the host, reducing it quickly into an empty capsule. It then evacuates the meconium, turns into a short, white prepupa, soon transforms itself into a slender, naked pupa in the host-tunnel and subsequently emerges as adult. The egg-period is one day, larval period 4.7 days, pupal period 5 to 7 days and the total life-cycle varies from 12—18 days. The maximum duration of life of the adults, when fed on raisin, was 13 days. The adult is not a strong flier and is difficult to breed in captivity as seen from limited trials in the laboratory. This parasite is subject to the attack of a hyper-parasite *Eupelmella pedatoria* Ferr. in the full grown larval and pupal stages.

Eupelmus sp. is another primary ectophagous parasite. It lays its eggs mostly on young grubs, although it sometimes chooses more advanced, medium-sized grubs also. Egg-period is not known and larval period occupies about five days, prepupal period one day and pupal period ranges from five to nine days. It was found only occasionally in very small numbers.

Eupelmus urozoñus Dalm. is an occasional ectophagous parasite of *Pempherulus* grubs. This species prefers full-grown host-grubs, but sometimes also attacks medium-sized ones. The egg-period occupies about one day; larval period six to eight days; prepupal period about one day; and pupal period from six to nine days averaging 7.5 days. The species also occurs in association with alternate host plants like *Sida acuta*.

Aplastomorpha calandrae (How.) sometimes occurs in association with alternate host plants like *Triumfetta rhomboidea*. It has been found to mate and oviposit in captivity. It also reproduces parthenogenetically giving rise to males. It lays its eggs in most cases singly after paralysing the host-grubs, which may be either medium sized or full grown. The pre-oviposition period varies considerably up to a maximum of 22 days. Egg-period is about one day, larval period four to six days, prepupal one to three days and pupal period varies from five to ten days. The total life-cycle ranges from 16 to 17 days.

Eupelmella pedatoria Ferr. is a wingless, shining dark Chalcid. It parasitizes not only *Pempherulus* grubs but also those of *Hypolixus truncatulus* and *Apion corchori*. It also functions as a hyper-parasite on larva and pupa of *Euderus*. Though not economically of much significance it is of considerable scientific importance due to its peculiar habits of reproduction and its double role as a primary and secondary parasite. The life-cycle varies from 17 days in July to 23 days in November, averaging 20.7 days during the period. The duration of life of the adult ranges from 6 to 47 days averaging 19.7 days for a dozen individuals. Three generations of the parasites have been reared in the laboratory without encountering any males. Probably males are unknown in the species. A detailed account of this species has already been published [Krishna Ayyar, 1940,1]. *not recd.*

Other species of parasites: An unidentified Chalcidoid. and a Braconid probably of the genus *Microbracon* have been, on rare occasions, taken from

Pempherulus in cotton. The Braconid has also been actually reared in the laboratory from parasitized host-grubs collected from the field.

PARASITES IN ASSOCIATION WITH ALTERNATE HOST PLANTS

It may be stated that as the existence of alternate host plants was its doubted at the commencement of this scheme, parasites from this source were totally unknown. When parasites were collected from these food plants, the studies imparted a new orientation to the problem of biological control of *Pempherulus*. Nearly a thousand parasites belonging to different species were collected from *Triumfetta rhomboidea*, *Corchorus olitorius*, *Sida acuta*, *Sida glutinosa*, *Malvastrum coromandelianum* and *Hibiscus esculentus*. Among these, those secured from *Triumfetta* formed the great bulk (Tables X and XI). Particular interest has attached to the incidence of parasites since the ultimate aim of this work is to find measures for the control of the weevil. The following species have been bred from this source :—

Chalcidoidea—

1. *Entedon pempheridis* Ferr.
2. *Dinarmus sauteri* Masi*
3. *Eupelmus urozonus* Dalm.
4. *Euderus pempheriphila* Ramkr. and Mani

Braconidae—

5. *Spathius labdacus* Nixon
6. *Spathius critolaus* Nixon
7. *Rhaconotus cleantes* Nixon
8. *Rhaconotus menippus* Nixon

Besides these, a Nematode parasite, *Geomermis indica* Steiner has also been noted. Five of these species, namely, *Entedon pempheridis*, *Dinarmus sauteri*, *Spathius labdacus*, *Rhaconotus cleantes* and *Rh. menippus* are absent in cotton fields. Most of the species are new to science and have only been recently described.

Seasonal incidence

The data collected on this aspect are presented in Tables X and XI. Although the parasites occur throughout the year, their maximum incidence seems to be from September to November, which period synchronizes with the early stages of the first brood of *Pempherulus*. If any of these could establish in cotton, it may be able to keep the pest under control. In fact, a single collection of two species of these parasites was actually made from cotton fields, when the infested alternate food plants were spread in the cotton crop.

Biology of the parasites

It has not been possible to study the life-histories of all the species. A separate account, embodying all available information has already been published [Krishna Ayyar, 1940,1], brief summaries of which are furnished below :—

Entedon pempheridis Ferr : This species is totally absent in cotton fields although it is the most widely distributed and most numerous among alternates.

* *Dinarmus coimbatorensis* Ferr., recorded in previous papers is a synonym of *sauteri* Masi.

TABLE X

Seasonal incidence of parasites in nature from alternate host plants based on data collected every month from different localities

Parasites	1937-38										1938						Per- cent- age of each kind				
	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Per- cent- age of each kind	Apr.	May	June		July	Aug.	Sept.	Oct.
<i>Eutadon pemphle- rite</i>	1	...	1	1	23	103	73	51	23	4	13	21	56.8	4	27	30	33	27	13	43	42.4
<i>Spathius labda- cus</i>	2	16	26	24	32	3	7	23	24.0	...	20	34	18	11	26	38	34.2
<i>Dinarmus sau- teri</i>	1	16	8	8	16	11	3	...	7	2	13.0	2	12	13	14	9	8	10	15.8
<i>Rhaconotus clean- thes</i>	10	1	2.0	...	5	5	8	...	3	4	5.8
<i>Spathius crilo- tus</i>	2	3	2	...	1	3	2	2	2.7	1	0.3
<i>Euderus pem- pheriphila</i>	1	1	0.35	1	0.3
<i>Aplastomorphus calandras</i>	1	1	...	0.35	1	0.3
<i>Eupelmus uro- zonus</i>	1	...	0.2
Doubtful cases	2 Br.	1	0.6	1	2	0.9
Total	4	...	2	17	34	130 (A)	130 (B)	87 (C)	59	11	31	48	100.0	6	64	85	73	48	55	97	100.0

(A) *Spathius crilolus* from *Melochia* excluded, (B) 1st record of Nematode parasite, (C) 2nd record of Nematode parasite

TABLE XI
Alternate host plants—consolidated summary, 1936-38

Plant species	1936				1937				1938			
	Total No. of plants examined	Percent- age of infesta- tion	Average percent- age of parasitism	Total parasites recovered	Total No. of plants examined	Percent- age of infesta- tion	Average percent- age of parasitism	Total parasites recovered	Total No. of plants examined	Percent- age of infesta- tion	Average percent- age of parasitism	Total parasites recovered
<i>Triumfetta rhomboidea</i>	2,362	55.8	8.9	423	2,637	81.9	12.0	415
<i>Sida acuta</i>	1,772	9.5	2,652	14.5	3.3	18	692	43.5	5.1	15
<i>Sida spinosa</i>	93	24.7	42	22.9	19.0	8	25
<i>Melastrium coromandelianum</i>	1,432	20.7	1,829	14.0	1.8	2	1,346	9.9
<i>Corchorus olitorius</i>	143	14.7	878	34.0	2.9	14	498	18.3
<i>Melochia corchorifera</i>	163	7.4	8.0	1	95

plant parasites. It has been bred from *Pempherulus* infesting *Triumfetta rhomboidea*, *Sida acuta*, *Corchorus olitorius*, *Malvastrum coromandelianum*, also an *Apion* grubs boring into *C. olitorius*. It is a primary larval parasite and the only endophagous species so far noted on *Pempherulus*. A single egg is usually laid inside the host-grub and the larva on hatching consumes the contents of the host. Just prior to pupation it issues out of the empty tunnel and pupates in the tunnel. Since parasitism by this species is the highest, it is likely to prove very useful.

Dinarmus sauteri Masi : It is a primary ectophagous parasite of *Pempherulus* grubs. Its life-cycle covers a period of 17-21 days. It has been collected on *Pempherulus* attacking *T. rhomboidea*, *Sida acuta*, *Corchorus olitorius* and *Phaseolus esculentus*. It is absent in cotton fields.

Spathius labdacus Nixon : It is a large, spotted-winged species, with a long ovipositor. It has been bred only in association with one wild food plant, namely, *T. rhomboidea*. It is a primary, ectophagous parasite, choosing its victims from among the healthy non-parasitized full-grown grubs. Its life-cycle ranges from 18 to 22 days. It has been successfully reared in the laboratory. Parthenogenetic reproduction is common.

Rhaconotus cleantes Nixon and *R. menippus* Nixon : These slender Ichneumonids are more or less similar in appearance and habits. They have been bred in association with *T. rhomboidea*, *C. olitorius* and *Sida acuta*. These are primary and ectophagous. These attack full-grown host-grubs. The life-cycle roughly occupies 16 to 24 days. These parasites are entirely absent in cotton fields.

Other species of parasites such as *Euderus pempheriphila*, *Eupelmus spurius*, *Spathius critolaus*, etc. have already been reviewed under cotton field-parasites.

Nematode parasite (*Geomermis indica* Steiner) : This generally infests cotton stem grubs boring into *T. rhomboidea* and feeds on the internal fluids. It has been identified by Dr Steiner as *Geomermis indica*. The genus itself is known only from U. S. A. so far.

REPORTED PARASITES

Over 20 different species of parasites numbering in all about 600 individuals consisting mostly of Braconids and Chalcidoids obtained from different lots of infested plant material were collected in the course of the north Indian tour. The entire collection comprised parasites either from cotton stem-borers or other stem-borers belonging to allied families. Attempts were made to breed them on *Pempherulus* grubs. Among these, only a few species were seen to possess possibilities of utilization. One of these is a species of *Spathius* parasitizing *Dinoderus* in bamboo, collected from Jawalapur (United Provinces) and the other, a larger Braconid—*Doryctes* sp.—attacking stem-borers (undetermined) boring into *Milletia* at Dehra Dun.

Spathius vulnificus Wlkn. from Jawalapur : This is a winged form of *Spathius*, closely resembling the local species (*S. critolaus* Nixon) in size and general appearance. Being a larval parasite, its potential efficiency is great. It searches out and follows up the grubs of *Pempherulus* and *Hypolixus*, and is applied to them in stems in cages. Its fecundity is 89 eggs. As many as 89 eggs have been seen to be deposited on a single grub of *Hypolixus*, which is capable of supporting all these to maturity. This heavy super-parasitism

though an apparent advantage for mass-breeding, is wasteful in the field. life-history has been worked out. The total life-cycle varied from 19 to 25 days, made up of an egg-period of two days, larval period of six days, pupal period of six days and a pupal period varying from 6 to 13 days. Males emerge one or two days earlier than females. The pre-oviposition period ranged from 4 to 20 days. The duration of life extended up to three months. Since it admitted of rearing in the laboratory, mass-breeding was attempted. It was more or less encouraging in the beginning. It, however, showed a tendency to a gradual decline in numbers from generation to generation. It may be easier to obtain thousands of these parasites by importing parasitic material from the original source where it is plentifully available.

Doryctes sp. from *Millettia* : This species is a comparatively large Braconid which freely develops on *Hypolixus* and *Pempherulus* grubs provided inside stems in cages. It is capable of laying a maximum of 27 eggs. The total life-cycle occupies about 24 days with an egg-period of two days, larval period of six to seven days, prepupal period of about two days and a pupal period of 11—13 days. The pre-oviposition period varies from 4 to 21 days. The egg-laying capacity is comparatively poor. Mass-breeding was attempted but without much success.

MASS-BREEDING, EXPERIMENTAL RELEASES AND RECOVERIES

The main attempt at mass-breeding centred round *Spathius critoides*. The imported species, *Spathius vulnificus*, also received some attention.

Spathius critoides : Availability of host material is one of the chief factors in mass multiplication. *Pempherulus* stages are only available during cotton season and even then only in small quantities ; during the rest of the year it breeds on two alternate hosts.

Hypolixus truncatulus and *Sinoxylon sudanicum* : The former host was utilized for rearing parasites inside the laboratory in small cages and the latter was useful for breeding in large out-door cages. The Bostrychid—*Sinoxylon*—generally bores into wilting and wilted Cambodia cotton stalks ; occasionally it also bores into living plants in the field. The female tunnels into the stem and constructs an enlarged chamber for pairing, into which the male enters. After mating, the female makes a new side tunnel, where it deposits the eggs. The grubs, on hatching, make long galleries along the stem and pupate in galleries filled with wood-dust and excreta. The adult beetles emerge by boring their way out of the stems. The entire life-cycle occupies roughly six to seven weeks. The adults can be easily collected during the afternoon, especially in November and December. There appear to be four distinct broods. The mature grubs of this borer form the preferred hosts of the parasite. In the case of *Hypolixus*, eggs are laid in cavities hollowed out in stems which are later on sealed. The average egg-laying capacity is about 50 eggs per female, with the maximum rising up to 78. It passes through five larval instars before turning into a prepupa. The total life-cycle averaged 42.7 days within a range of 35—55 days. The egg-period averages about 4.6 days, the larval 24 days and the prepupal and pupal together about 13 days. The duration of life of the adult female averaged 42.6 days and that of the male 38.5 days. The study of this host has an added significance in the present studies. It is a heavily parasitized insect in nature with a set of

4 different species of parasites. As many as five or six species among these also parasitic on *Pempherulus* in cotton.

By a judicious handling of these two hosts about 9000 parasites were usually reared during the period. Table XII furnishes data on their numbers and seasonal occurrence.

TABLE XII
Spathius critolaus (1936-1938)

Month	Collections from outdoor cage			Rearing in laboratory cage		
	1936	1937	1938	1936	1937	1938
January	76	39
February	55	26	10
March	274	53	81	..	32	19
April	185	90	116	60	16	1
May	7	288	101	355	10	13
June	9	182	295	62	31	..
July	264	159	710	19	11	..
August	385	117	873	5	30	25
September	279	301	565	180	29	50
October	685	324	541	183	8	65
November	365	232	..	20
December	179	260	..	33	9	..
Total	2,632	2,137	3,321	917	202	183

It may be noticed from Table XII that the emergence of the parasites was greatest during July to October, which is a very convenient time for releasing them in the fields to control the first generation of the pest. The sex-ratio of the parasite shows a slight preponderance of females averaging 53 per cent from *Bostrychid* hosts and 58 per cent from *Hypolixus*. About 5-6 per cent of these are winged forms.

Releases

The release of this species was attempted twice in the field cages during the period. The first of these was vitiated by the following unforeseen cause and therefore could not be pursued. The plants grown in cages for the purpose

during the off-season were heavily covered by aphids and coccids with swarms of attendant ants. The ants had their nests so thickly honey-combed in and around these cages that their control was found impossible. The parasites liberated were destroyed by the ants before they could settle down on plants for parasitization. A second trial was made in another cage. Though this proved better, it suffered from another type of unexpected handicap, the plants being grown in a field cage had to be artificially infested with weevils. The weevils were not available for the purpose during October due to the absence of any infested cotton crop in the vicinity. Therefore, adults of the parasite from alternate host plants like *Triumfetta* were introduced into the cages for infestation. It was found later that such adults (though of the same species) did not readily take to cotton; and only very poor oviposition was taken place with the consequent scarcity of suitable grub stages in the plants at the time of parasite releases. Pest infestation and parasite releases were, however, carried on continually for some more time. All plants that indicated weevil attack externally were pulled out and examined for parasites. The results obtained, despite the handicap, were of sufficient significance.

TABLE XIII

Parasite releases and recoveries

Month	No. of plants examined	Percentage of plants attacked	Percentage of parasitism based on		Number of parasites liberated	Remarks
			Pest stage	Total infestation		
November 1937 .	12	91.7	58.3	38.9	121	Live and dead stages only 5 all
December 1937 .	12	91.7	20.0	6.3	48	
January 1938 .	14	100.0	21.4	17.6	23	
February 1938 .	6	100.0	60.0	33.3	4	
Total .	44	95.9	31.5	22.1	196	Average percentage infestation and parasitism

The data recorded above show that the percentage of parasitism of live and dead stages varied from 20—60 per cent with an average of 31.5 per cent for the entire lot. The live stages were rare due to light infestation. This level of recovery has to be deemed encouraging in the case of a stem borer. It is clear that provided adequate releases are made at

time under favourable conditions, the parasite will work efficiently. Experiments, however, call for more trials in view of the many points in the biology of this parasite. Its life-cycle is only a fourth of the period taken by the pest and it can, therefore, complete not less than three generations by the time the host completes one. It is not wasteful in egg-laying and it chooses healthy grubs. The average egg-laying capacity of the parasite (22-24) is nearly equal to that of the host (about 24 eggs). The sex-ratio is nearly 1:1 which will enable the parasite easily to overtake the pest. It has no alternate host-parasite and can tide over off-seasons by living on the two alternate hosts. Besides, it can by itself live long in nature. It occurs in most localities where the pest is found and at a critical time when the first brood of the pest is hatching. The low parasitism of 1 per cent recorded under field conditions may be ascribed to a multiplicity of factors. A certain proportion of hosts may be inaccessible due to their concealed habits. This is overcome to some extent by the presence of a long ovipositor in the parasite. Another is the restriction of victims being restricted to medium and mature host-grubs and it may be that only a fraction of the grubs are sufficiently far advanced for their parasitism. This is partly got over by the prolonged grub period and the slow development of the hosts which makes the host-grubs available almost throughout the season. Again, the host-grubs lie scattered in different plants and at distances. They can be reached by the small proportion of winged parasites. It can also be remedied artificially by large releases. Notwithstanding these advantages it is possible that its biotic potential may be low under field conditions.

The imported species, *Spathius vulnificus*, was, as stated already, amenable to rearing in the laboratory. At one time a fair number of adults was available and a small number (about 77 consisting of 71 females) was liberated in the cotton field but no recoveries could be made.

III. PRESENT POSITION OF THE PROBLEM AND CONCLUSIONS

The present investigation, covering a short period of three years, has not reached a stage when the possibilities or otherwise of controlling *Pemphorus* and its natural enemies can be definitely declared. Its biology, geographical distribution, alternate food plants, original home and natural enemies have been studied. The first infestation of cotton is due to weevils, emerging from its alternate hosts and wild food plants near cultivated areas. It is noted that the pest has only recently become a major pest of cotton in south India which probably began to attack about 25—30 years ago. Previous to this, the pest probably confined its attack to wild food plants like *Triumfetta rhomboides*, *Sida acuta*, etc. and its numbers are supposed to have been controlled by the action of the set of parasites associated with them in this wild habitat. The introduction and extensive cultivation of Cambodia cotton and its consequent expansion in areas and intensity resulting in vast 'monocultures' added the weevil with a new domain, having an inexhaustible supply of hosts.

Its conditioning in this variety gradually adapted it for attacking other varieties, such as country cottons. The weevil's habits have also adjusted themselves to the altered environment. The parasites of the weevil in its original and wild habitat failed to accompany the same successfully into the

cotton fields. But the abundance of weevils in cotton may not be due to the absence of parasites. A partial study of the physical ecology of weevils suggests that extremes of climatic conditions in south India are sufficiently great to offer an effective check on its multiplication as in parts of India. It looks as if a series of years of severe drought may cause a serious reduction in the population of the weevil by the partial destruction of its wild host plants. On the other hand, a series of seasons with heavy rain would appear to produce more favourable conditions for its increase.

Experiments on pests like stem-borers necessarily require a good many years before definite results emerge. A considerable volume of precise knowledge on these aspects has, however, been accumulated now but these have only covered the essential preliminary stages. Many vital aspects of the biology of the pest still await investigation. The studies of alternative food plants of the weevil have reached a stage when the main problem of host-attraction and nutrition can be proceeded with. A further study of the food sites discovered in association with cotton and other food plants has not yet been made. The exact relation of the few known enemies to the pest and the individual rôles in the host-parasite complexes call for further study. Particular attention may be directed to two lines of investigation which seem of special interest. Mass-multiplication of parasites associated with the pest and their liberation at suitable times may effect some measure of control. The second is the colonization of parasites (associated with alternative food plants) in cotton fields. The percentage of parasitism in nature is low but is important since this small force of parasitic element is an essential factor in maintaining an equilibrium in nature. It is reasonable to suppose that the conservation, multiplication, and liberation of parasites in fields, will at least act as an auxiliary agent in pest-control.

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In conclusion, the writer takes this opportunity to record his thanks to the Indian Central Cotton Committee for financing the scheme, to the Cotton Specialist and the members of his section, who have afforded him help in one form or another in carrying out the investigation during the past three years. He also wishes to place on record the enthusiastic co-operation he has received from the Assistants and Fieldmen, who have been associated with him during the three years of the scheme. He is indebted to Sir Guy Marshall and the specialists of the British Museum for the identification of weevils and parasites.

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ON THE NATURE OF REACTIONS RESPONSIBLE SOIL ACIDITY

VIII. THE ACID CHARACTER OF HYDROGEN CLAY IN RELATION TO SOME PROBLEMS OF SOIL SCIENCE*

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IN parts V—VII of this series [Mitra, 1936; Mitra, Mukherjee and Bagchi, 1940; Mitra, 1940] several features of the acid character of hydrogen clays have been discussed. Parts V and VI mainly dealt with the variations of the total neutralizable acid, that is, the base exchange capacity, under different conditions, and the characteristics of the titration curves have been discussed in part VII. The following aspects of the relation of the acid character of hydrogen clay with some problems of soil science are discussed in this part:—

1. Regular, specific and mixed cation effects in the interaction of hydrogen clay with neutral salts and bases.
2. The liberation of aluminium from hydrogen clay by neutral salts.
3. The role of cation effects in the estimation of the base exchange capacity of hydrogen clays and soils.
4. Variations in the form of the titration curves of entire hydrogen clays and hydrogen bentonite fractions of several Indian soils and bentonites.
5. Variations in the properties of sub-fractions of the entire hydrogen clay fraction of a soil.
6. Alterations in the properties of hydrogen clay on the removal of inorganic oxides contained in it.

* The results given in this paper have been taken from the published Annual Report for 1934-35, 1935-36, 1936-37, 1937-38, and 1938-39 on the working of a scheme of research into the 'Properties of Colloid Soil Constituents' financed by the Imperial Government of Agricultural Research, India.

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† Bentonites are formed by the weathering of volcanic rocks and have chemical composition similar to that of soil. They are known to contain clay minerals belonging to the montmorillonite group and thus form an important link in the chain of systems lying between simple substances such as SiO_2 , Al_2O_3 , Fe_2O_3 and standard clay minerals on the one hand and the very complex hydrogen clays on the other.

EXPERIMENTAL

The method of preparation of the hydrogen clays and hydrogen bentonites experimental procedure have been described in part IV [Mukherjee *et al.*,], and parts V and VII. Particulars regarding the soils and bentonites and the hydrogen clays and hydrogen bentonites which were obtained from them are given in Table I.

TABLE I
Particulars regarding the soils and bentonites used

	Description of soil or bentonite*	Silica-sesquioxide ratio (molar) of entire clay fraction	Reference number of corresponding hydrogen clay or hydrogen bentonite
3	Brownish yellow soil (unmanured) from Government Farm, Suri (Bengal) collected at a depth of 6-12 inches from Agricultural Chemist's experimental plot, block A 1-16, plots Nos. 3, 5, 16	2.34	E
4	Highland acid soil from Government Farm, Burdwan (Bengal) collected at a depth of 0.6 inches from block B, plot No. 40 of the Farm	1.94	F
20	Neutral calcareous soil (brown loam) from Government Seed Farm, Kalyanpore (U. P.) collected at a depth of 0.6 inches	2.10	H
25	Black cotton soil (neutral, calcareous) from Satara (Bombay), collected at a depth of 0.6 inches	2.50	I
32	Neutral black soil from Bilaspur near Raipur (C. P.), collected at a depth of 0.6 inches	2.54	K
22	Red lateritic soil (acidic) from Government Farm, Dacca (Bengal) collected at a depth of 0.6 inches	1.99	L
34	Black soil from Government Farm, Akola (Berar) collected at a depth of 0.9 inches	2.19	M
33	Bhata red laterite soil from C. P. collected at a depth of 0.9 inches	1.88	N
46	Non-lateritic calcareous soil (B-type) from Government Farm, Padegaon (Nira, Poona) collected at a depth of 0.12 inches	2.51	Padegaon-B
51	Acid soil from Government Farm, Jorhat (Assam), collected at a depth of 0.6 inches	2.58	Jorhat-F
53	Highland acid soil on old alluvium from Government Farm at Latekujan (Assam), collected at a depth of 0.6 inches	2.47	Latekujan-F
D.	Bentonite from Hati-Ki-Dhani	2.86	Hati-Ki-Dhani-B
D.	Bentonite from Bhadres	2.90	Bhadres-B
3			

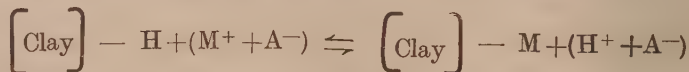
* The samples of bentonite were kindly supplied by the Assam Oil Company.

RESULTS

1. *Regular, specific and mixed cation effects in the interaction of hydrogen clay with neutral salts and bases**

In parts V and VI [Mitra, 1936 ; Mitra *et al.* 1940] it has been shown that the total neutralizable acid of a hydrogen clay sol, called in agricultural science the base exchange capacity (b. e. c.), is, unlike acids in true solution, a fixed quantity but depends on cation effects, the pH and, in some cases, on the time allowed for the interaction with the base. The higher the pH, the larger is the b. e. c. The cation effects are illustrated by : (a) the dependence of the b.e.c. calculated at the inflexion point and, more strikingly, at a fixed pH (e.g. pH 7.0), on the cation of the base ; (b) the much larger b.e.c. obtained on titration in the presence of, or on leaching by, neutral salts than with the base alone ; and (c) the differences observed between the effects of various cations of neutral salts. In the absence of salts the b.e.c. decreases in the order $\text{Ca}(\text{OH})_2 > \text{Ba}(\text{OH})_2 > \text{NaOH}$ which, however, changes to $\text{Ba}(\text{OH})_2 > \text{Ca}(\text{OH})_2 > \text{NaOH}$ in the presence of a fixed concentration of the corresponding chlorides. The differences in the relative effect of Ba^{++} and Ca^{++} ions have been traced to the fact that in the presence of BaCl_2 the greater part of the reaction with the base usually takes place between pH 3.5 and 5.5 ; whereas, when no salt is present, the reaction is mainly confined within the range of pH 5.5-6.5. In the presence of the salts the cation effect is regular in the sense that it follows the lyotrope series which is determined by the order of electrical adsorption of cations together with their hydration envelopes [Mukherjee, 1922]. At the comparatively higher pH values which obtain in the absence of the salts, the cations are probably adsorbed in a dehydrated state which would account for what has been called the irregular cation effect operating under these conditions.

The mixture of the sol and salt contains H^+ ions some of which are present in the intermicellary liquid and others† are associated with the colloidal particles or flocs. The incomplete displacement of the H^+ ions by the cations of the salt is to be referred to the balanced nature of the reaction which can be represented according to the following simple scheme ignoring complicating factors :



where M^+ and A^- are respectively the cation and the anion of the added salt. The intensity of the back reaction is determined by the total concentration of H^+ ions in the liquid. When a hydrogen clay is repeatedly leached with a salt solution, the H^+ ion concentration of the salt extract rapidly decreases as the leaching proceeds, thus favouring more and more the direct reaction. The process is also very much enhanced if the salt solution is a buffer having a fixed pH. In the interaction with a base the back reaction is almost absent, securing a more complete replacement of the H^+ ions by the cations.

* The work described in this section was carried out by Mitra along with others. Some results have been given in part VI.

† Aluminium ions appear to be present in the double layer in addition to hydrogen ions (see sub-section 2). Apart from this, the nature of the primarily adsorbed anions and the crystal structure of the particles require to be considered in detail.

Other peculiarities of cation effects, not discussed in the previous parts of this series and not clearly recognized by previous workers, have also been recorded in this paper. They are observed when the added salt has cations other than those of the base used for the titration. Such mixed cation effects are of interest. The soil absorption complex usually contains more than one type of exchangeable cations and the part they may play, individually and relatively to each other, on the base exchange and other reactions of the complex is not definitely known. Ionic antagonism effects are well known in colloidal behaviour [Freundlich and Scholz, 1922; Mukherjee and Ghosh, 1924]. A study of the mixed cation effects may also throw light on observations such as that of Renold [1936] who found that a mixed permutite, e.g. a K-Ba-permutite prepared from a Ba-permutite by treatment with a K-salt, has not the same base exchange property as another having an identical composition and amount of exchangeable Ba^{++} and K^+ ions but prepared from a K-permutite by interion exchange with a Ba-salt.

The cation and the pH effects are illustrated below.

TABLE II

Base exchange capacity in m. e. base per 100 gm. of oven-dried hydrogen clay using NaOH, Ba(OH)₂, and Ca(OH)₂

System	NaOH		Ba(OH) ₂		Ca(OH) ₂	
	At inflex. pt.	At pH 7.0	At inflex. pt.	At pH 7.0	At inflex. pt.	At pH 7.0
E	2.2 (5.4)*	15.4	20.6 (6.0)	25.0	21.5 (5.8)	26.2
E+0.1N BaCl ₂	28.0 (4.6)	>42.4
E+0.1N CaCl ₂	21.2 (4.4)	40.6
E+0.1N NaCl	16.1 (5.0)	26.4
Padegaon B	57.0 (7.4)	53.5	89.0 (8.05)	74.0	91.0 (8.02)	80.0
Padegaon-B+0.002 N NaCl	70.0 (8.0)	63.0	78.0 (8.0)	67.0	80.0 (8.0)	69.0
Padegaon-B+0.002 N BaCl ₂	55.0 (5.7)	68.0	67.0 (6.1)	70.0	63.0 (6.1)	70.0
Padegaon-B+0.002 N CaCl ₂	52.0 (5.63)	67.0	67.0 (5.66)	70.0	60.0 (6.05)	69.0
Padegaon-B+0.10 N NaCl	85.0 (7.53)	80.0	90.0 (7.2)	87.0	90.0 (7.46)	85.0
Padegaon-B+0.10 N BaCl ₂	65.0 (5.26)	114.0	74.0 (5.0)	122.0	70.0 (5.1)	116.5
Padegaon-B+0.10 N CaCl ₂	63.5 (5.30)	110.0	72.5 (5.15)	120.0	70.0 (5.1)	116.5

* The figures in brackets denote the pH at the inflexion point of the titration curve.

The variations of the b. e. c. recorded in Table II are summarized below

Experiment	Variations of b. e. c. observed	Inference
1. Sol titrated with different bases	$\text{Ca(OH)}_2 > \text{Ba(OH)}_2 > \text{NaOH}$	Irregular, or specific cation effect
2. Mixture of sol and salt titrated with the corresponding base	<p>(a) $\text{Ba(OH)}_2 > \text{Ca(OH)}_2 > \text{NaOH}$ at inflexion point of E and at pH 7.0 of both E and Padegaon-B</p> <p>(b) $\text{NaOH} > \text{Ba(OH)}_2 > \text{Ca(OH)}_2$ at inflexion point of titration curves of Padegaon-B</p> <p>(c) The inflexion point gives a smaller b. e. c. of Padegaon-B and BaCl_2 (or CaCl_2) mixture compared with Padegaon-B itself</p>	<p>Regular cation effect</p> <p>The apparent order $\text{Na}^+ > \text{Ba}^{++} > \text{Ca}^{++}$ is to be referred to much higher pH at inflexion point in the titration curve of mixture of sol and NaCl compared with the mixture containing BaCl_2 (or CaCl_2). The pH masks the cation effect</p> <p>The regular cation effect is masked by the pH effect as the inflexion point in the titration curve of the mixture occurs at a much lower pH than that of the sol</p>
3. Mixture of Padegaon-B and a fixed conc. of NaCl, BaCl_2 or CaCl_2 titrated with different bases	$\text{Ba(OH)}_2 > \text{Ca(OH)}_2 > \text{NaOH}$	The regular cation effect. The smaller b. e. c. of the mixture of sol and 0.1 N BaCl_2 or CaCl_2 obtained on titration with NaOH than with Ba(OH)_2 or Ca(OH)_2 indicates some sort of an antagonism between the comparatively few Na^+ ions of NaOH and the large number of Ba^{++} or Ca^{++} ions of the salts. The strong adsorption of Ba^{++} and Ca^{++} ions and their capacity to displace H^+ ions from the double layer appear to be somewhat inhibited by Na^+ present at a much lower concentration
4. Mixture of Padegaon-B and a fixed conc. of different salts titrated with the same base	<p>(a) $\text{BaCl}_2 > \text{CaCl}_2 > \text{NaCl}$ at pH 7.0</p> <p>(b) $\text{NaCl} > \text{BaCl}_2 > \text{CaCl}_2$ at the inflexion point of the titration curve</p>	<p>Regular cation effect</p> <p>Regular cation effect is masked by the pH effect</p>

2. *The liberation of aluminium from hydrogen clay by neutral salts†*

Aluminium ions are known to be set free by the interaction of neutral salts with acid soils and hydrogen clays [Paver and Marshall, 1934]. There is no difference of opinion regarding the nature of the reaction by which the aluminium is liberated. A direct exchange of the Al^{+++} ions by the cations of the added salt has been suggested by some workers [Daikuhara, 1914; Popen, 1916] while others [Page, 1926; Wilson, 1929] consider that aluminium is brought into solution by a secondary dissolution of aluminium oxide by the acid generated on the addition of a salt. An exchange of both H^+ and Al^{+++} ions by the cations of the salt has also been postulated [Paver and Marshall, 1934].

The possible sources of these displaced Al^{+++} ions are: (i) free Al_2O_3 contained in the hydrogen clay, (ii) Al^{+++} ions inside the lattice of the mineral constituents of the clay and (iii) Al^{+++} ions present on the surface in (a) a primary or (b) secondarily adsorbed condition. Aluminium in all these three forms may react with acids and bases. Toxic properties of acid soils have often been attributed to aluminium found in the soil solution. There is evidence to show that Al^{+++} ions are stable on the surface of colloidal particles of aluminium oxide sols at a pH as high as 6.0 [Mukherjee *et al.*, 1932; also unpublished work of B. Majumdar in this laboratory].

In part VII of this series it has been shown that at concentrations below 0.02N alkali metal cations liberate practically no aluminium from hydrogen clays and consequently an exchange of H^+ ions against the cations of the salt has to be postulated to account for the titratable acid of the neutral salt extract. The subject has been studied in detail by one of us (B. Chatterjee). While a detailed account will be published separately, some of his results are given below.

Increasing amounts of BaCl_2 were added to hydrogen clay H. Table III illustrates the relations between (i) the amounts of Al liberated, (ii) the total acidity of the clear supernatant liquid above the coagulum of the sol and salt mixture obtained on centrifuging this mixture in resistance glass containers, and (iii) the amount of Ba adsorbed by the hydrogen clay.

TABLE III

Relation between the Al liberated, the total acidity of the supernatant liquid and the Ba adsorbed by the hydrogen clay

(50 c. c. of the sol taken for each experiment; time of interaction 24 hours)

Concentration of added BaCl_2	m. e. per 100 gm. colloid		
	Displaced Al	Displaced acid	Adsorbed Ba
0.01 N	3.1	11.4	11.1
0.02 N	4.9	11.9	12.7
0.04 N	8.2	14.4	15.2
0.09 N	12.6	17.0	18.5
0.20 N	22.0	22.0	24.0

† The work discussed in this section was carried out by Chatterjee with the help of others. Details will be published in a separate series of papers.

As the concentration of the salt increases more Al is liberated and concentration of 1.0 N, the liberated Al, the adsorbed Ba and the total acid all have almost identical values. A fair agreement between the total acid and the quantity of Ba adsorbed is observed at all concentrations of the BaCl_2 . It appears from the results that an exchange of both H^+ and Al^{+++} ions for the Ba^{++} ions takes place. With increasing salt concentration Al^{+++} ions are exchanged and the exchange of the two ions does not seem to be independent, although at very low concentrations of the salt only H^+ ions are exchanged.

The view that Al^{+++} ions are liberated by direct exchange and not by a secondary dissolution is in harmony with the fact that the curves (I) obtained on plotting (a) m. e. of Al liberated, (b) the free and total acids in the clear supernatant liquid and (c) the amount of Ba adsorbed, against the concentration of the added BaCl_2 , all have the form of the usual adsorption therm.

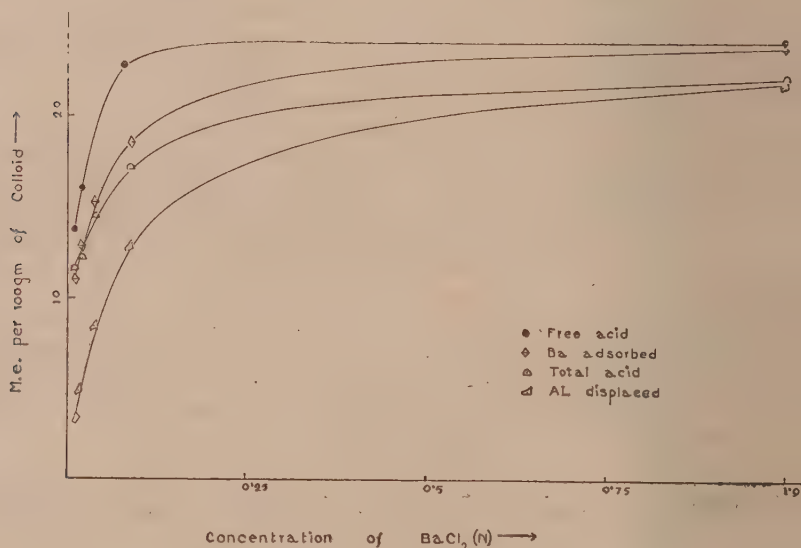


FIG. 1 Curves showing the relation between the Al liberated, the free and total acids of the supernatant liquid and the amount of Ba adsorbed

The above view is further supported by the following results obtained with Padegaon-B which show that the amount of aluminium liberated is materially the same, both when the pH of the sol is allowed to decrease with addition of the salt as also when the pH is kept constant by the use of a suitable buffer.

If the aluminium found in the supernatant liquid were dissolved from hydrogen clay by the acid set free, larger quantities would have been liberated when the buffer was not used. At constant pH also the amount of displaced Al steadily increases with the concentration of BaCl_2 .

TABLE IV

Al liberated from hydrogen clay with and without the addition of buffer

With buffer*			Without buffer		
Conc. of salt	pH	M. e. Al displaced per 100 gm.	Conc. of BaCl ₂	pH	M. el A displaced per 100 gm.
10 N BaCl ₂ + 0.016 N Na-Ac.	3.60	20.0	0.10 N	2.57	20.9
0 N BaCl ₂ + 0.02N Na-Ac.	3.64	40.9	1.0 N	2.54	40.9

* Sodium acetate + acetic acid.

*The role of cation effects in the estimation of the base exchange capacity of hydrogen clays and soils**

The base exchange capacity of a soil is an extremely ill-defined quantity [Hissink, 1935] and concordant results are seldom obtained by different routine methods used for estimating it [Crowther and Martin, 1925]. The uncertainty mainly arises from the difficulty of an unequivocal definition of the amount of reactive or exchangeable hydrogen (and aluminium). The variations of this quantity are capable of being accounted for by cation effects formulated by us and the equilibrium pH of the solution. The time of attainment of equilibrium is also of importance, especially in these systems where interaction of interfaces or inner surfaces [Wiegner, 1935] are involved. It follows from theoretical considerations [Mukherjee *et al.*, 1925] that if the concentration of cations is high the relative differences observed between them should become smaller. When the pH is high, its effect may even override the cation effect as previously shown. In other words, with a sufficiently high concentration of cations and of hydroxyl ions the difference in the b. e. c. obtained using different salts should be less and a definite limiting value would be obtained indicating the total amount of reactive hydrogen and aluminium ions which are probably present at different affinity levels and remain associated with the particles of the hydrogen clay. It is assumed that additional complicating factors, such as the dissolution of the particles and exposure of inner layers, are absent. A comparative study of some routine methods of estimating the b. e. c. has been made and the results including those published previously [Mitra and Mitra, 1940] are given in Table V.

Parker's [1929] and Schollenberger's [1930] methods give nearly the same b. e. c. At pH 7.0 and normal concentration, the difference between NH₄⁺ and Ca⁺⁺ ions vanishes. In the titration in presence of N BaCl₂ up to pH 7.0 the H and cation effects are comparable to what obtain in the above two methods and the values obtained by these three methods mutually agree. Schofield's

* The work discussed in this section has been carried out by Mitra and Mukherjee (S. K.) with the help of others and details are being published in a separate series of papers. Part I of this series has appeared [Mitra and Mitra, 1940].

method [1933] gives nearly the same result for Latekujan F but about cent higher value for the other hydrogen clay. More marked differences have been observed in the case of soils.*

TABLE V

B. e. c. in m. e. per 100 gm. of oven-dried (105°C.) hydrogen clay obtained by different methods

Hydrogen clay	Titration† with baryta in presence of <i>N</i> BaCl ₂	Parker's†† method	Schollen- berger's‡ method	Scho- field's‡‡ method	Estimation of cations adsorbed on leaching with neutral normal solutions of		
					N ₂ HCl	BaCl ₂	CaCl ₂
Jorhat-F	33.0	33.0	32.0	35.0	34.0	22.0	22.0
Latekujan-F	56.5	54.0	55.0	55.0	56.0	42.0	42.0

† To mixtures of the hydrogen clay and *N* BaCl₂ contained in a series of Jena glass bottles increasing amounts of Ba(OH)₂ are added; the mixtures are thoroughly shaken and kept overnight; the pH is measured on the following day and the b. e. c. is calculated from the inflexion point of the titration curve.

†† Ba adsorbed on leaching with neutral normal solution of barium acetate is displaced on further leaching with a neutral normal solution of NH₄Cl and estimated.

‡ NH₄ adsorbed on leaching with neutral normal solution of ammonium acetate is estimated.

‡‡ The amount of lime taken up from a half-neutralized solution (pH 7.1) of *p*-nitrophenol with this method is estimated.

The somewhat higher value obtained by Schofield's method can be traced to the following factors:—

(a) in this method the equilibrium pH is somewhat higher (7.1) than what is more important, the system is always maintained at this pH whereas in Parker's and Schollenberger's methods it has been found that a considerable portion of the total leaching solution used has a pH near about 6.4 after it has percolated through the hydrogen clay or soil; the pH rises slowly to 7.0 after the leaching has been continued for some time;

(b) a longer time** (16-18 hrs) of interaction is allowed in Schofield's method than in the other methods where the leaching is usually finished within 6 hrs;

(c) the greater adsorption of Ca⁺⁺ ions compared to Ba⁺⁺ and Na⁺ ions near about pH 7.0 in agreement with the irregular cation effect.

Ba⁺⁺ and Ca⁺⁺ ions are adsorbed from their neutral normal solution in amounts which are smaller than the b. e. c. determined by the method of Parker, Schollenberger and Schofield (Table V). The amount of NH₄ adsorbed from a neutral normal solution of NH₄Cl, however, is in fair agreement with this b. e. c. In the methods of Parker and Schollenberger the acetates are used as a buffer so that leaching proceeds near about pH 7.0. When barium calcium chloride is used the pH of the medium has been found to be near about 6.4, i.e. below 7.0 even after leaching with 500 c. c. of the solution, which accounts for the smaller amounts of Ba and Ca adsorbed from their chlorides. Using NH₄Cl, however, the pH rises up to 6.8 and this is partially, if not wholly, responsible for the apparently greater effect of the monovalent Na⁺ ions.

* Unpublished work of Mr S. K. Mukherjee.

** It has been found by Schofield [1933] that on allowing a still longer time of interaction a somewhat higher value is obtained,

Variations in the form of the titration curves of the entire hydrogen clay and hydrogen bentonite fraction of several Indian soils and bentonites†

The more general features of the titration curves of hydrogen clays prepared from the entire clay fractions have been discussed in part VII of this series and are briefly stated below: The different strong bases give titration curves having different forms. The potentiometric titration curves with silicic alkalis indicate a weak monobasic acid character (discussed below) with an inflexion point which lies between pH 's 7.2 and 8.5. The alkaline earth hydroxides, on the other hand, give curves resembling those of a strong monobasic acid. The strong or weak acid character, however, is only apparent and the titration curves reveal several features not ordinarily expected of acids in true solution. For example, the potentiometric and conductometric titration curves with a given base offer entirely conflicting evidence regarding the strength of the acid. In contrast to the weak acid character the potentiometric caustic alkali curves the corresponding conductometric curves show a sharp minimum indicative of a strong acid. On the other hand, the alkaline earth hydroxides give a conductometric curve with a flat rounded minimum suggesting that a weak acid is being titrated, while as stated above the corresponding potentiometric curve resembles that of a strong acid. In part VII, these features, difficult to understand from the classical electrochemical standpoint, have been reconciled in the light of the theory of the electric double layer and of adsorption of ions as postulated by one of us [Mukherjee, 1921, 1922]. Apart from these and other features of the titration curves which are common to the hydrogen clays we have studied, there are features which are different for different hydrogen clays. Reference to some of these has been made in part VII. These differences are very likely to be useful in the characterization and classification of the soils [Anderson and Byers, 1936] and are more fully discussed below. An error may easily be made in forming any conclusion regarding the acid character of hydrogen clays in general in the absence of observations on a sufficiently large number of them prepared from soils of widely different origin and type. Moreover, the properties of the entire clay fraction is an integral of those of the particles of different sizes of which it is composed and a study of the sub-fractions should be of great help in the classification of soils. Our work on the sub-fractions has been discussed in the next section.

Figs. 2, 3, 4 and 5 illustrate the different types of potentiometric titration curves. The curves given in the figures were obtained on titrating hydrogen clays Latekujap-F, Padegaon-B, F, M, N and K and the hydrogen bentonites Hati-Ki-Dhani-B and Bhadres-B.

The NaOH curves are of three different types:

(a) To the first type belong the titration curves of the hydrogen clay and the hydrogen bentonite Hati-Ki-Dhani-B given in Fig. 2. The curves resemble those of a dibasic acid. They have an initial strong acid character and a weak inflexion in the acid region. In these respects there is a resemblance with silicic acid sols [Chatterjee, 1939]. Further studies regarding the significance of this dibasic character are under way. The second inflexion occurs in the neutral to weakly alkaline region. Silicic acid sols do not show an inflexion in this region of pH .

† This work was carried out by Mitra,

(b) The second type of dibasic NaOH curves is illustrated by those of Latekujan-F also shown in Fig. 2. They have an initial weak acid character. Similar types of curves were obtained on titrating a hydrogen kaolinite* prepared from a sample of the mineral from Singbhum (Bengal).

(c) To the third type of NaOH curves given in Fig. 3, belong those of the majority of hydrogen clays studied by us. The titration curves given in this figure are those of hydrogen clays Padegaon-B and N and the hydrogen bentonite Bhadres-B. The curves resemble those of a weak monobasic acid.

The $\text{SiO}_2 : \text{R}_2\text{O}_3$ ratio of the hydrogen clays showing the first two types of curves are 2.47 and 1.94. The hydrogen clays showing the third type of curves have $\text{SiO}_2 : \text{R}_2\text{O}_3$ ratios 1.88 and 2.51. It is apparent that this ratio which represents the mass chemical composition does not determine the form of the titration curve.

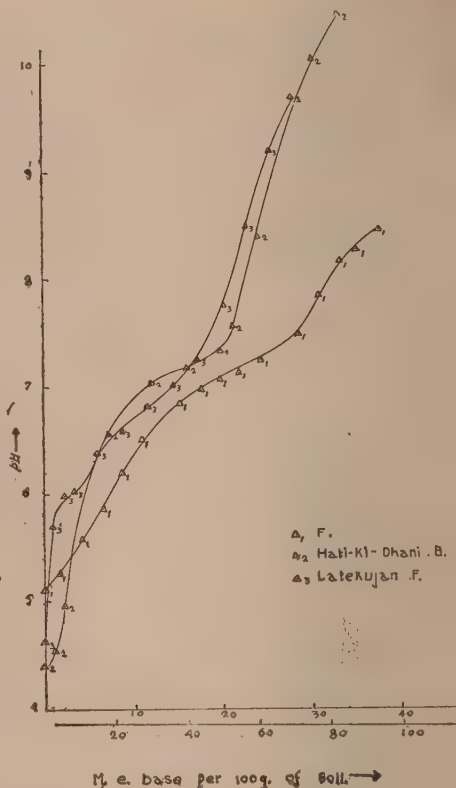


FIG. 2. Potentiometric titration curves of hydrogen clay and hydrogen bentonite having a dibasic acid character

The $\text{Ba}(\text{OH})_2$ and $\text{Ca}(\text{OH})_2$ curves given in Figs. 4 and 5 are of the following types :

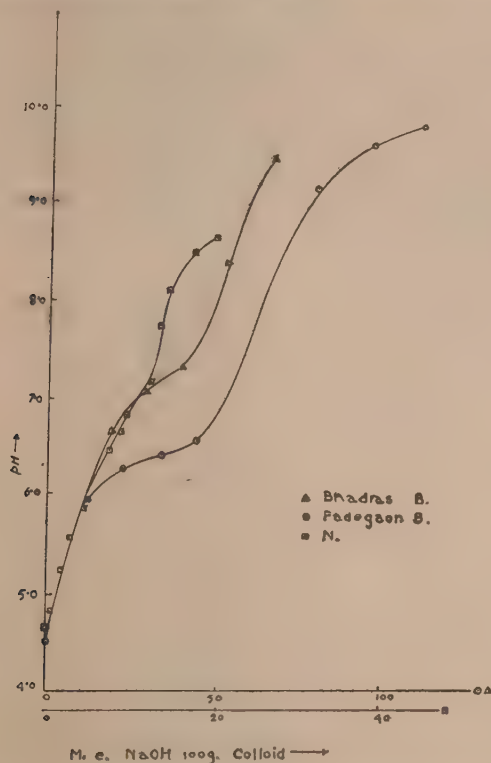
(a) N and Bhadres-B show an initial rise followed by a buffering characteristic of weak acids.

(b) The second group shows a comparatively flat initial run followed by a more or less sharp inflexion given by strong acids (Padegaon-B and N). The majority of the hydrogen clays studied by us show titration curves of this type. Differences are observed in the sharpness of the inflexion point and its location in the pH scale. The inflexion point usually lies between 5.5 and 6.3 and, in a few cases, between pH's 6.3 and 7.0.

(c) Hati-Ki-Dhani-B shows a strong dibasic acid character which has been observed by us so far with any hydrogen clay when titrated with alkaline earth hydroxides.

* Unpublished work of Mitra.

(d) The fourth group shows a definite lowering of pH in the initial stages of the titration, e.g. the titration curves of M. This peculiar feature which a simple explanation is difficult to suggest has also been observed in the titration curves of sub-fractions of M.



3 Potentiometric titration curves with NaOH of entire hydrogen clay and hydrogen bentonite fractions having a weak monobasic acid character

These observations are of a novel nature and are worth following up in soil. The complexities we have observed compel the conclusion that our present notions about the acid character of clays and soils and mineralogical X-ray analyses independent of electrochemical studies are not adequate for scientific purposes.

*Variations in the properties of sub-fractions of the entire hydrogen clay fraction of a soil**

The entire clay fraction consists of soil particles of all sizes below 2μ . According to recent work the fractions containing particles of different sizes do not always have the same chemical and mineralogical composition or

* This work has been carried out by Mitra. Details will be given in a separate series of papers.

base exchange capacity [Marshall, 1935]. The identification of the constituents of the sub-fractions is of considerable interest and several well-known physical methods, e.g. X-ray, thermal and optical analyses have been requisitioned for this purpose. Valuable information may be obtained through the application of the electrochemical technique including a comparison of the inflexion points and forms of the titration curves of the different fractions and their base exchange capacities calculated per gramme (T_g per sq. cm. (T_s) of the external surface. Its importance has not so far been recognized. Its usefulness may be further increased by similar studies on standard clay minerals.* About 40 sub-fractions of typical Indian clays have been examined by us with this object in view. The relation between particle size and the electrochemical properties of colloidal solutions is a subject of considerable theoretical interest.

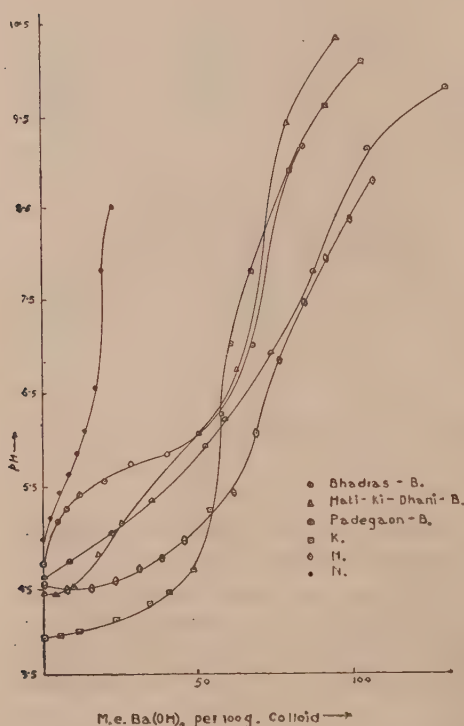


FIG. 4 Different types of potentiometric titration curves with $Ba(OH)_2$ of entire hydrogen clay and hydrogen bentonite fractions

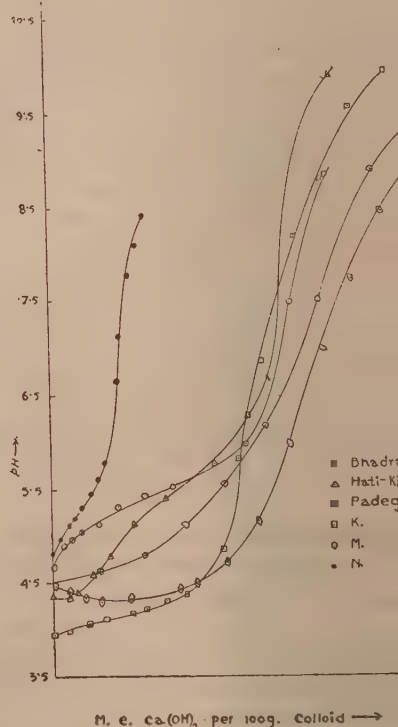
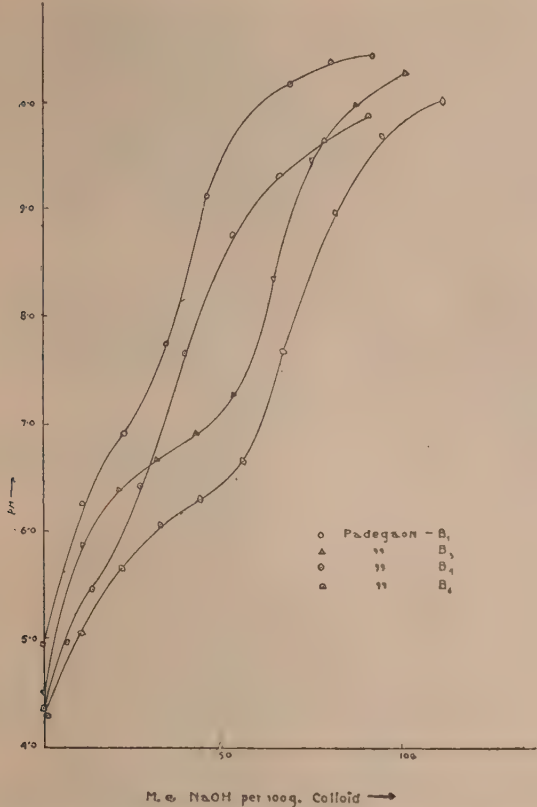


FIG. 5 Different types of potentiometric titration curves with $Ca(OH)_2$ of entire hydrogen clay and hydrogen bentonite fractions

The particle sizes, chemical compositions and b. e. c.'s (T_g and T_s) calculated from the titration curves with $NaOH$ of six sub-fractions of the hydrogen clay fraction of the black cotton soil from Padegaon have been given in Table VI, and the titration curves of four of them in Fig. 6.

* The properties of clay minerals are being studied by Mitra.



6. Potentiometric titration curves with NaOH of sub-fractions of hydrogen clay isolated from the Padegaon soil

TABLE VI

Chemical composition and base exchange capacity of sub-fractions of hydrogen clay isolated from the Padegaon soil

Reference No. of sub-fraction	Mean equivalent spherical microns	Chemical composition on the ignited basis			Base exchange capacity	
		SiO ₂ per cent	Al ₂ O ₃ per cent	Fe ₂ O ₃ per cent	M. e. per 100 gm (T _g)	M. e. per sq. cm. of surface × 10 ⁷ (T _s)
1	1.1	59.3	19.8	12.8	46.5	230.0
2	0.15	56.3	21.7	17.0	59.5	40.0
3	0.07	59.5	21.6	14.5	63.0	20.0
4	0.03	64.5	18.0	11.4	63.0	9.6
5	0.018	66.7	17.5	11.0	70.0	5.5
6	<0.015	60.0	26.8	10.5	40.0	<2.3

The sub-fractions were obtained by graded centrifugalization—a S supercentrifuge was used—of the entire clay following Ayre's procedure described by Whitt and Bayer [1937]. In calculating the particle size of the external surface the same density of the different fractions and a spherical symmetry of the particles were assumed. The variations in properties of the sub-fractions with diminishing particle size may be summed up as follows:

Property	Variations
1. Chemical composition—	
(a) Percentage of SiO_2	Increases ignoring fractions 1 & 6
(b) Percentage of Al_2O_3	Decreases ignoring fractions 1 & 6
(c) Percentage of Fe_2O_3	Decreases ignoring fraction 1
2. Base exchange capacity—	
(a) T_s	Increases except for fraction 6 which has the smallest T_s
(b) T^a	Decreases
3. Form of titration curves	No marked variations with the particle size except of fraction 6

The variations in chemical composition may arise from (a) isomorphous replacements [Marshall, 1935 ; Hendricks *et al.*, 1930] within the lattice of constituent minerals, (b) differences in relative proportions of several of clay minerals and/or inert materials, e.g. 'free' silica and sesquioxide.

The fact that the different fractions give more or less the same type of titration curves with the possible exception of fraction 6 indicates that they contain essentially the same acid material. The isomorphous replacements mentioned above would probably give rise to appreciable differences in the features of the curves.

The variations in T_s may be referred, at least in part, to differences in the chemical composition. Fractions 4 and 5, however, have nearly the same chemical composition and the variations of T_s in their case do not admit of such explanation. The increase in T_s of these fractions with the particle size signifies that the reaction with the base is not confined to the external surface alone but fresh layers are continuously exposed as the action with the base proceeds and/or the particles have considerable internal surfaces where the reaction takes place.

6. Alterations in the properties of hydrogen clay on the removal of free inorganic oxides contained in it*

The inorganic colloidal material of soil is associated with varying amounts of 'free' oxides of Si, Al and Fe. A comparative study has been undertaken of the changes in (i) the chemical composition, (ii) the nature of titration curves with bases, and (iii) the b.e.c.'s calculated from them consequent on treatments aiming at the removal of these free oxides. The methods described by previous investigators are not free from the criticism that the

*This work has been carried out by Mitra along with others. Details will be published in a separate series of papers.

compose or alter the nature of the exchange complex proper and may effect a complete separation of the free oxides. It is of interest to compare the changes brought about by them. Results* given below illustrate these changes.

The hydrogen clay L from the red lateritic soil from Dacca was treated by the method of Truog *et al.* [1936]. The b. e. c.'s calculated at the inflexion points of the titration curves of L and its derivative L_d obtained after the treatment have been given in Table VIII and the results of fusion analysis in Table VII.

TABLE VII

Chemical composition of the hydrogen clay from the Dacca lateritic soil before and after removal of free inorganic oxides

Hydrogen clay	Chemical composition on the ignited basis		
	SiO ₂ per cent	Al ₂ O ₃ per cent	Fe ₂ O ₃ per cent
L	51.2	36.0	12.0
L_d	57.5	38.0	5.7

TABLE VIII

b. e. c. of hydrogen clay from Dacca lateritic soil before and after removal of free inorganic oxides

Hydrogen clay	B. e. c. in m. e. base at inflexion point of titration curve with		
	NaOH	Ba(OH) ₂	Ca(OH) ₂
L	16.3 (8.2)	17.5 (7.1)	19.0 (6.8)
L_d	24.5 (9.5)	23.5 (9.0)	25.0 (9.1)

The figures in brackets denote the pH values at the inflexion points. The b. e. c.'s of L_d have been calculated at the second inflexion point (see below).

The alterations in properties consequent on the removal of the free oxides are very significant. The b. e. c. of L_d is definitely greater than that of L and shows that inert materials having negligible b. e. c. have been removed. The chemical composition of L_d approaches that (SiO₂—55.45 per cent; Al₂O₃—45.5 per cent) of kaolinite if allowance is made for some isomorphous replacement of Al by Fe. The titration curves of L_d (Fig. 7)

*Obtained with the help of Sankarananda Mukherjee.

with all three bases have the same form and reveal a weak dibasic character, a feature also shown by kaolinite**. The titration curves with three bases of L, on the other hand, have markedly different forms. Further, the b. e. c. of L_d at the second inflexion point is very nearly equal to that of kaolinite. The observations on the whole, indicate that kaolinite is the dominant mineral constituent of the clay fraction of the Dacca laterite

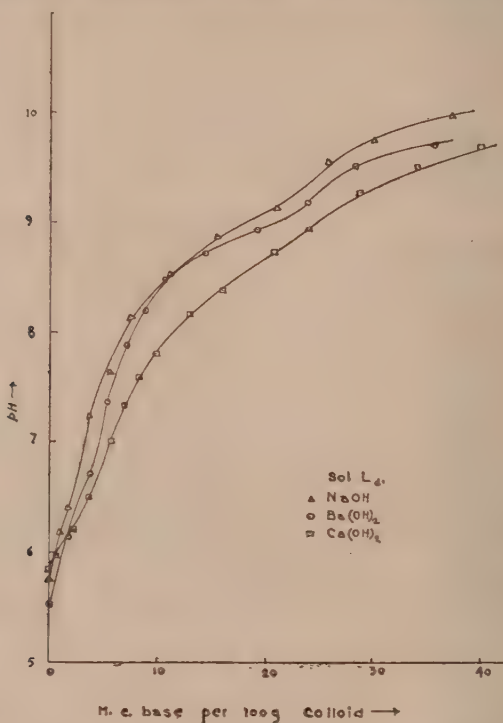


FIG. 7. Potentiometric titration curves of hydrogen clay from Dacca lateritic soils after removal of free inorganic oxides

SUMMARY

1. The base exchange capacity (b. e. c.) of a hydrogen clay is not a fixed quantity but depends on the pH and cation effects and in some cases on the time allowed for the interaction with the base. The higher the pH, the larger is the b. e. c. The cation effects are illustrated by (a) the dependence of the b. e. c. calculated at a fixed pH on the cation of the base used for titration, (b) the much larger b.e.c. obtained on titration in the presence of neutral salts than with the base alone and (c) the differences observed between the effects of various cations of neutral salts. In the absence of salts,

**Unpublished work of Mitra (R. P.) and Mitra (D. K.).

***They show the following features: NaOH curve: weak monobasic; Ba(OH)₂ and Ca(OH)₂ curves: strong monobasic. (See section 4).

follows the order $\text{Ca}(\text{OH})_2 > \text{Ba}(\text{OH})_2 > \text{NaOH}$ which illustrates an ionic or specific cation effect in that the relative effects of the Ca^{++} and Ba^{++} ions are in violation of the lyotrope series. In the presence of a fixed concentration of the corresponding chlorides the order changes to $\text{Ba}(\text{OH})_2 > \text{Ca}(\text{OH})_2 > \text{NaOH}$ and the cation effect is regular. The difference between the relative effects of the Ca^{++} and Ba^{++} ions in the two cases has been traced to the fact that in the presence of the salts the greater part of the interaction of the base takes place at a much lower $p\text{H}$, usually between 3.5 and 5.5, when no salt is added. In the latter case, the reaction is mainly confined within the range of $p\text{H}$ 5.5 to 6.5.

2. The b. e. c. of several hydrogen clays has been estimated by the methods of Barker, Schollenberger, Schofield and by titration with $\text{Ba}(\text{OH})_2$ in the presence of N BaCl_2 and the results discussed in the light of the $p\text{H}$ and ionic effects.

3. Both H^+ and Al^{+++} ions are exchanged for the cations of a neutral salt on interaction with a hydrogen clay. With increasing salt concentration more and more Al^{+++} ions are exchanged although at very low concentrations of the salt only H^+ ions are exchanged. The quantity of Al exchanged on the addition of a fixed concentration of the salt is materially the same when the $p\text{H}$ decreases on the addition of the salt as also when it is kept constant by the use of a suitable buffer.

4. Differences have been observed in the form of the titration curves of hydrogen clays prepared from the entire clay fractions of several Indian soils and their importance in the classification and characterization of the soils has been discussed. The NaOH curves are of three types: weak monobasic, which is the most common; weak dibasic; and strong dibasic. The $\text{Ba}(\text{OH})_2$ and $\text{Ca}(\text{OH})_2$ curves are of four types each: strong monobasic, the most common type; strong dibasic; weak monobasic; and strong monobasic but showing an actual turning of the $p\text{H}$ on the addition of the base in the initial stages of the titration.

5. Hydrogen clays prepared from six sub-fractions of the entire clay fraction of an Indian black cotton soil give nearly the same type of titration curves. With diminishing particle size, the base exchange capacity calculated per gramme increases except for the finest fraction but calculated per sq. cm. of the external surface, the b.e.c. diminishes.

6. Marked alterations in the base exchange capacity, chemical composition and form of titration curves of a hydrogen clay prepared from the entire clay fraction of an Indian laterite soil have been observed consequent on the removal of its free silica and sesquioxides by the method of Truog and coworkers.

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INTERACTION BETWEEN HYDROGEN CLAYS AND NEUTRAL SALTS

I. THE NATURE OF THE INTERACTION RESPONSIBLE FOR THE LIBERATION OF ALUMINIUM*

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WHEN a neutral salt is added to a hydrogen clay or an acid soil, an acid reaction is developed and the neutral salt extract often contains Al and Fe. There is no unanimity of opinion regarding the mechanism by which Al and Fe are brought into solution. Two theories have been put forward to explain the nature of the reaction. The one, advocated by Daikuhara [1914] and Jappen and coworkers [1916, 1921, 1926, 1929], suggests that Al and Fe are liberated as the result of a simple exchange of these ions by the cations of the added salt. The acid developed has been ascribed to the normal hydrolysis of the resulting Al- and Fe-salts. The other theory supported by Page [1926], Magistad [1925], Kelly and Brown [1926] and Mattson [1933] assumes that in this reaction the main replacement is one of H^+ ions by the cation of the added salt. The free acid thus formed dissolves aluminium and iron oxides contained in the soil or clay. Paver and Marshall [1934] have recently investigated the interaction between neutral salts and hydrogen clays. They consider that a direct exchange of both H^+ and Al^{+++} ions by the cations of the added salts takes place and they have postulated that a hydrogen clay is really a mixed clay, viz. H-Al-clay.

The methods for the preparation and purification of hydrogen clays and the general experimental arrangements used in this work for the estimation of neutralizable acid and the amounts of Ba^{++} adsorbed were the same as described in previous publications from this laboratory [Mitra, 1936 ; Mukherjee, *et al.*, 1937 ; Mitra, *et al.*, 1940 ; Mitra, 1940]. The amount of Al present in the neutral salt extract was estimated by means of 8-oxyquinoline using the method of Berg [1927]. The free sesquioxides were removed by the methods of Tamm [1922] and Truog, *et al.* [1936].

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The following soils were used for this work :

Description of soil	Silica-sesquioxide ratio (molar) of entire clay fraction	Reference number corresponding hydrogen clay
Neutral calcareous soil from Govt. Seed Farm, Kalyanpore (U. P.) collected at a depth of 0 — 6 in.	2.10	H
Red lateritic soil (acidic) from Govt. Farm at Dacca (Bengal) collected at a depth of 0 — 6 in.	1.99	L
Non-lateritic calcareous soil (B-type) from Govt. Farm at Padegaon (Nira, Poona) collected at a depth of 0 — 12 in.	2.51	Padegaon-B
Highland acid soil on old alluvium from Govt. Farm at Latekujan (Assam) collected at a depth of 0 — 6 in.	2.47	Latekujan-F
Black cotton soil (neutral, calcareous) from Satara (Bombay) collected at a depth of 0 — 6 in.	2.50	I

RESULTS AND DISCUSSION

Relation between the amount of displaced Al, the titratable acidity of the filtrate and the amount of cation adsorbed

Increasing amounts of BaCl_2 were added to hydrogen clay sols H and L and estimations were made of (i) the total acidity of the supernatant liquid above the coagula of the sol + BaCl_2 mixture, (ii) the amount of displaced Al and (iii) the amount of Ba adsorbed. The results are shown in Table I.

The sol and salt mixture was centrifuged after 24 hours from the time of adding the salt to the sol.

TABLE I

Amounts of Al displaced by BaCl_2 from sols H and L, the titratable acidity of the filtrate of sol + BaCl_2 mixtures as also the amounts of Ba adsorbed

Hydrogen clay	Conc. of BaCl_2	pH of mixture	pH of centrifugate	M.e. per 100 gm. colloid		
				Al* in supernatant liquid	Ba adsorbed	Total acidity of supernatant liquid
H	0.01N	3.41	3.57	3.1	11.1	11.4
	0.02N	3.37	3.50	4.9	12.7	11.9
	0.04N	3.38	3.52	8.2	15.2	14.4
	0.09N	3.36	3.35	12.6	18.5	17.0
	1.0N	3.19	3.33	22.0	24.0	22.0
L	0.02N	..	3.27	..	13.0	12.0
	0.04N	..	3.25	10.7	13.8	15.0
	0.09N	..	3.23	12.6	17.7	19.0
	1.0N	..	3.05	17.1	18.8	19.3

*Fe could not be detected in the supernatant liquid.

At low concentrations of the added salt the total acidity of the supernatant liquid (i.e. exchange acidity) cannot be wholly accounted for by the amount of Al present. With increasing concentration of BaCl_2 more and more Al^{+++} is liberated and at a concentration of $1.0N$ BaCl_2 , the liberated Al, the adsorbed Ba and the exchange acidity have nearly identical values. A close agreement between the total acidity of the supernatant liquid and the amount of Ba adsorbed is observed. The $p\text{H}$ values of the mixtures containing $0.02N$, $0.04N$, and $0.09N$ BaCl_2 do not differ widely but the amount of Al in the supernatant liquid steadily increases with the concentration of BaCl_2 . It appears that the free acid developed on the addition of neutral salts does not play a prominent role in the liberation of Al. A direct exchange between both Al^{+++} and H^+ ions for Ba^{++} ions offers a more plausible explanation.

Relation between the pH of the sol and salt mixture and the amount of displaced Al

Hydrochloric acid is generated in the interaction between the hydrogen peroxide and the added BaCl_2 . In order to examine the extent to which Al is liberated by free HCl, normal HCl was added drop by drop till its $p\text{H}$ became almost equal to that of 'sol and salt' mixture. The amounts of Al in the supernatant liquids above 'sol and HCl' mixtures are given in Table II.

TABLE II

Amounts of Al displaced by HCl and BaCl_2 respectively at almost same pH

Sol	pH of the mixture		M.e. Al in the supernatant liquid per 100 gm. colloid	
	Sol and salt	Sol and acid	Sol and salt	Sol and acid
.	3.19	3.07	22.0	1.9
.	3.0	3.0	17.1	1.0
degaon-B	2.54	2.52	40.9	7.5

At the same $p\text{H}$ the amount of Al brought into solution by HCl constitutes a small fraction of that liberated by BaCl_2 . By far the major portion of the Al liberated by the neutral salt cannot, therefore, be attributed to any dissolution of aluminium oxide by the free acid developed in the salt extract. Over and Marshall [1934] obtained similar results. They found that at equal strengths (normality) the amount of Al liberated by BaCl_2 is almost double of that liberated by HCl.

The addition of the salt to the sol lowers its $p\text{H}$. Experiments were carried out in which the concentration of BaCl_2 was gradually increased but the $p\text{H}$ was maintained practically constant by using a buffer. Sodium acetate-acetic acid buffer has been used and the results are given in Table III.

TABLE III

Effect of pH on the liberation of Al by BaCl₂ from sol Padegaon-B
 (pH of the sol (Padegaon-B) 3.70, colloid content 35.1 gm./l, time of interaction 24 hours)

System	With buffer			Without buffer		
	Conc. of salt	pH	M.e. Al per 100 gm. colloid	Conc. of BaCl ₂	pH	M.e. Al per 100 gm. colloid
25 c.c. sol . . .	0.04N BaCl ₂					
+ 23 c.c. buffer . . .	+	3.70	10.0	0.04N	2.61	
+ 2 c.c. N BaCl ₂ . . .	0.018N Na-Ac.					
25 c.c. sol . . .	0.10N BaCl ₂					
+ 20 c.c. buffer . . .	+	3.60	20.0	0.10N	2.57	
+ 5 c.c. N BaCl ₂ . . .	0.016N Na-Ac.					
25 c.c. sol . . .	1.0N BaCl ₂					
+ 25 c.c. buffer . . .	+	3.64	40.9	1.0N	2.50	
+ 6.1 gm. BaCl ₂ . . .	0.02N Na-Ac.					

Na⁺ ions are introduced into the system along with the buffer. The concentration, however, remains practically constant and variations in amount of Al liberated should be ascribed to the changing concentration of Ba⁺⁺ ions. Besides, the capacity of Na⁺ ions to liberate Al has been found [Chatterjee and Paul, 1942] to be very small compared with that of Ba⁺⁺ ions. Table III shows that the amount of displaced Al increases steadily with the concentration of BaCl₂. At any given concentration of BaCl₂, the amount of Al displaced is independent of the variation of pH observed with and without buffer. These observations support the postulate of a direct exchange of Al⁺⁺⁺ ions.

Na⁺ ions introduced along with the buffer may, however, give rise to certain complications. 'Ionic antagonism' is known [Freundlich, 1913; Freundlich and Scholz, 1922; Mukherjee and Ghosh, 1924; also unpublished work of Mitra] to play an important part in reactions in colloidal systems involving more than one type of ions carrying a similar charge. In order to avoid these complications, experiments were carried out in which the pH of the hydrogen clay and BaCl₂ mixtures was adjusted at a practically constant value (3.8) by adding the requisite amounts of Ba(OH)₂. The results obtained with sol Latekujan-F are given in Table IV.

TABLE IV

amounts of Al displaced by BaCl₂ from sol Latekujan-F at a constant pH as also when the pH is not adjusted with Ba (OH)₂

Sol + BaCl ₂			Sol + BaCl ₂ + Ba(OH) ₂		
Conc. of Ba ⁺⁺	pH	M.e. Al displaced per 100 gm. colloid	Conc. of Ba ⁺⁺	pH	M.e. Al displaced per 100 gm. colloid
0.16N . . .	3.70	0.63	0.00162N	3.8	1.0
0.32N . . .	3.54	2.2	0.0033 N	3.8	1.8
0.48 N . . .	3.40	4.3	0.0082 N	3.8	4.1
0.64 N . . .	3.30	5.1	0.0163 N	3.8	6.2
0.80 N . . .	3.07	15.0	0.0706 N	3.8	15.3

The concentration of Ba⁺⁺ ions does not increase materially on the addition of Ba(OH)₂. The amount of Al liberated at pH 3.8, however, increases with the concentration of the added BaCl₂ and it seems that the pH of the mixture is not of much consequence in determining the amount of liberated Al at a given concentration of Ba⁺⁺ ions.

Effect of removal of the free sesquioxides contained in the hydrogen clay on the quantity of Al displaced by neutral salts

If Al were liberated as a result of secondary dissolution of Al₂O₃ contained in the hydrogen clay, a decrease in the amount of Al liberated would be observed on the removal of the free sesquioxides by suitable methods. The free sesquioxides of hydrogen clay Padegaon-B were removed by the methods of Tamm [1922] and Truog [1936]. The amounts of Al liberated by 0.1N BaCl₂ before and after the removal have been compared in Table V.

TABLE V

amounts of Al displaced by 0.1 N BaCl₂ from sol Padegaon-B before and after the removal of free sesquioxides

System	M.e. Al liberated per 100 gm. colloid
Padegaon-B	20.9
Padegaon-B (after treatment according to Tamm's method) .	25.4
Padegaon-B (after treatment according to Truog's method) .	36.0

The results show that the amount of Al liberated is not reduced on the removal of the oxides. On the contrary, an increase (calculated per 100 gm. of the residual colloid) is observed. This increase is in agreement with the assumption that the amount of active material per 100 gm. increases on the

removal of free oxides which are really inert instead of being the source of liberated Al. This observation is in agreement with the observed increase in the base exchange capacity of hydrogen clay sols on the removal of the sesquioxides by the method of Truog *et al.* [unpublished work of M. Truog]. When Tamm's method was used the changes in the base exchange capacity were irregular. With some sols an increase was observed, while others showed a decrease. This necessitates a systematic study on the effect of the removal of the free sesquioxides on the displacement of aluminium. Further work on this topic is in progress.

Effect of time on the amounts of (i) the cation adsorbed, (ii) the exchange acidity and (iii) the Al liberated

Kappen [1929] observed that the reaction between an acid soil and a neutral salt proceeded so quickly that the pH measured at definite intervals since the beginning of the reaction showed no material variations. This has been used by Kappen as an evidence against the secondary dissolution of Al. The idea of an exchange adsorption mooted by Kappen has been contradicted by Page [1926] who is of opinion that 'there is in the liquid phase in contact with the soil absorptive material, at any given degree of unsaturation of the latter, an equilibrium concentration not only of hydrogen ions, but also of aluminium hydroxide, and that both these concentrations increase together.' Paver and Marshall [1934] observed that the Al liberated was most equivalent to the exchange acidity for shorter periods but showed a distinct fall later. This decrease in the Al liberated was accompanied by a fall in the pH. They consider that in the later stages a small amount of aluminium hydroxide was being adsorbed and a corresponding amount of acid liberated. In the light of their results the effect of time on the amounts of (i) Ba adsorbed, (ii) the exchange acidity and (iii) liberated Al has been studied with hydrogen clay sols H and I using 0.09N BaCl₂. The results are given in Table VI.

TABLE VI

Variations with time in the amounts of (i) the cation adsorbed, (ii) the exchange acidity and (iii) the liberated Al in the case of sols H and I using 0.09 N BaCl₂

Sol	Time allowed	pH of the centrifugate	M.e. Al in the centrifugate per 100 gm. colloid	M.e. Ba adsorbed per 100 gm. colloid	M.e. exchange acidity per 100 gm. colloid
H (SiO ₂ /R ₂ O ₃ = 2.1)	5 mins.	3.28	12.5	18.1	18.1
	30 "	3.06	15.6	20.6	23.1
	6 hours	3.14	13.6	19.9	19.9
	24 "	3.33	12.6	18.6	17.6
	48 "	3.30	12.2	18.5	17.6
I (SiO ₂ /R ₂ O ₃ = 2.5)	5 mins.	2.75	26.4	35.1	34.1
	30 "	2.74	27.5	34.9	32.1
	6 hours	2.70	26.9	..	34.1
	24 "	2.70	26.1	32.0	34.1

With sol H the Al liberated, adsorbed Ba and the acid displaced all increase at first. A decrease is then observed and finally they become constant. These results are in agreement with those obtained by Paver and Marshall [1934] who, however, measured only the variations of the amount of Al displaced and the pH of the salt extract. The amount of the cation adsorbed and the acidity developed were not estimated. Their observations do not show the initial increase in the amount of Al displaced. The data cited in Table VI show in addition that the decrease in the amount of Al displaced is not accompanied by a fall in pH nor with any increase in exchange acidity. And it appears that the assumption made by Paver and Marshall [1934] of a subsequent desorption of a small quantity of aluminium hydroxide resulting in the liberation of a corresponding amount of acid is not adequate. A decrease in the amounts of (i) displaced Al, (ii) adsorbed Ba and (iii) the acid displaced in the later stages suggests that after some time some re-adsorption of Al^{+++} ions takes place accompanied by a 'desorption' of the adsorbed Ba^{++} ions. With sol I no material variations in (i), (ii) and (iii) with time were noticed. This observation is in agreement with that of Kappen [1929]. It appears that the discrepancy in the results obtained by different workers probably arises from the use of different types of soils. The electrochemical properties of sols H and I have also been found to differ in several important points [Mitra, 1940].

SUMMARY

1. The acid liberated on the addition of a neutral salt to a hydrogen clay cannot be wholly accounted for by the amount of Al present in the salt extract when low concentrations of the salt are used. The amount of Al displaced increases with the concentration of the salt.
2. The titratable acid of the $BaCl_2$ extract and the amount of Ba adsorbed by the hydrogen clay are in fair agreement.
3. At the same pH, the amount of Al brought into solution by HCl constitutes a small fraction of that liberated by $BaCl_2$. Practically the same amount of Al is liberated when both the pH of the sol decreases as the result of the addition of the salt as also when the pH is kept constant by the use of a suitable buffer, or by adding the requisite amount of the corresponding base.
4. The amount of Al displaced does not decrease on the removal of free sesquioxides contained in the hydrogen clay but increases.
5. The time that elapses after addition of the salt to the sol has some effect on the amount of Al found in the salt extract. The time effect appears to be influenced by the type of soil from which hydrogen clay is obtained.

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THE ROLE OF ALUMINIUM IN RELATION TO THE FREE AND TOTAL ACIDS OF HYDROGEN CLAYS*

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(With six text-figures)

INVESTIGATIONS with hydrogen clay sols in this laboratory [Mitra, 1936 ; Mukherjee *et al.*, 1937 ; Mitra *et al.*, 1940 ; Mitra, 1940] show that : (i) the free acidity of a hydrogen clay sol, calculated from the *pH* value usually constitutes a small fraction (3-10 per cent) of its total neutralizable acid, determined from the inflexion point in the titration curve with a base, (ii) the amounts of acid which interact with different bases are in the order $\text{Ca}(\text{OH})_2 > \text{Ba}(\text{OH})_2 > \text{NaOH}$, (iii) the total neutralizable acid of a hydrogen clay sol is greatly increased on the addition of neutral salts, (iv) the total acidity of the supernatant liquid above the coagula of the 'sol and salt' mixture is considerably less than that of the suspension as a whole or that of the pure sol, and (v) the amount of acid displaced into intermicellary liquid depends on the electrical adsorbability of cations which is determined [Mukherjee, 1921, 1922] by their mobility and valency.

In the previous part [Mukherjee and Chatterjee, 1942] it has been shown that both exchangeable H^+ and Al^{+++} ions are present on the surface of the colloidal particles. The present investigation has been undertaken with a view to obtaining definite information regarding the role of these Al^{+++} ions on the free and total acids of a hydrogen clay sol.

The methods of preparation and purification of the sols and the experimental procedure have been described by Mukherjee and Chatterjee [1942].

RESULTS AND DISCUSSION

Figures of the titration curves of the sol, of the 'sol and salt' mixtures and their clear supernatant liquids

The potentiometric titration curve (Fig. 1) of the sol Latekujan-F with HCl reveals a weak dibasic acid character. The $\text{Ba}(\text{OH})_2$ and $\text{Ca}(\text{OH})_2$ curves (Figs. 2 and 3) on the other hand, resemble that of a weak monobasic acid. The form of the curve changes when a salt has been added and with

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increasing concentrations of the salt the form becomes progressively characteristic of a strong acid. The flocculation caused by salts leaves, after some time, a clear supernatant liquid whose titration curves (Figs. 4, 5 and 6) have forms widely differing from those of the sol and salt mixtures. The inflection portion becomes steep and merges into a region of noticeable buffering which becomes prominent with increasing concentration of the salt. The buffering occurs between pH 3.75 and 5.0 and merges in its turn into a second steep portion showing an inflexion point characteristic of the neutralization point of an acid or a base. These features have been observed with solutions of aluminium salts [Britton, 1927].

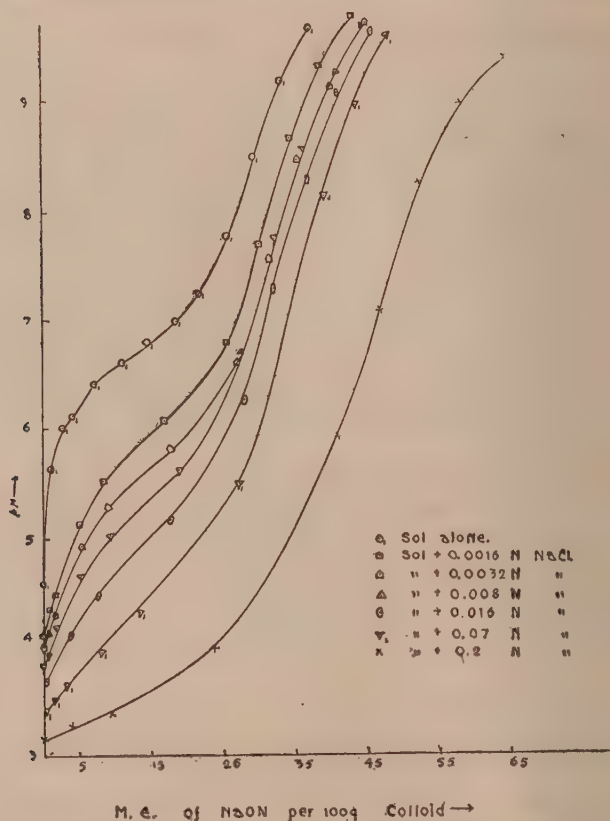


FIG. 1. Potentiometric titration curves of the sol Latekujan-F with NaOH

Relation between the total acidity of the hydrogen clay and salt mixture, the acidity of the supernatant liquid and the amount of Al displaced

Table I records the total reacting acid of the sol Latekujan-F, of its mixtures and with salts having six different concentrations of NaCl and of the supernatant liquids and the quantities of displaced Al in the supernatant liquids. Similar data for sol H and its mixtures with different concentrations of $BaCl_2$ are also given in the same table.

TABLE I.

acidities of sols Latekujan-F and H and salt mixtures and those of their supernatant liquid and the amounts of displaced Al

	Concentration of salt (N)	M.e. total acid* per 100 gm.				M.e. Al displaced per 100 gm.	Average	a-b	a-c	c-d
		Sol + salt	Sol	Super-natant liquid	Average					
		a	b**	c	c	d	d			
n-F	0.0016 NaCl	30.0	28.0	0.18 0.22	0.20	NH		2.0	29.8	+0.2
	0.0032 "	31.5	"	0.32 0.28	0.30	0.34 0.30	0.32	3.5	31.2	-0.02
	0.008 "	32.0	"	0.62 0.58 0.60	0.60	0.62 0.55	0.58	4.0	31.4	+0.02
	0.016 "	32.5	"	1.8 1.9	1.85	1.20 1.25	1.22	4.5	30.6	+0.63
	0.070 "	34.5	"	4.1		2.68 2.60	2.64	6.5	30.4	+1.47
	0.20 "	44.0	"	10.0		11.0		16.0	34.0	-1.0
n-F	0.0016 BaCl ₂	31.0	30	1.4 1.4	1.4	0.66 0.61	0.63	1.0	29.6	+0.77
	0.0032 "	32.0	"	3.2		2.1 2.2	2.15	2.0	28.8	+1.05
	0.008 "	38.0	"	4.8		4.3		3.0	28.2	+0.5
	0.016 "	34.5	"	7.0		5.1		4.5	27.5	+1.9
	0.070 "	42.0	"	16.0		15.0		12.0	26.0	+1.0
	0.20 "	50.0	"	22.4		19.0		20.0	27.6	+3.4
	0.01 BaCl ₂	33.0	28.5	11.0		3.0 3.2	3.1	4.5	21.6	+8.3
	0.02 "	35.0	"	12.0		4.9		6.5	23.0	+7.1
	0.04 "	37.6	"	14.4		8.2		9.0	23.1	+6.2
	0.09 "	43.0	"	17.0		12.6		14.5	26.0	+4.4
	1.0 "	44.0	"	22.0		22.0		15.5	22.0	0

* Calculated at the inflexion point in the titration curve with corresponding bases.

** Calculated at the second inflexion point in the titration curve with NaOH.

the total acidities decrease in the following order: 'sol and salt' mixture (ii) > supernatant liquid of the sol and salt mixture (iii).

Excepting the lower concentrations of NaCl* in the case of sol Latekujan- the highest concentration of BaCl₂ in the case of sol H, the total acidities of (iii) are definitely greater than their Al contents. This excess is due to the difference between the values under c and d in Table I where it

At the lower concentrations of NaCl the difference between the two quantities is within the limits of experimental error. The same holds for 0.2N NaCl and the two acidities are exactly equal at a concentration of 1.0N BaCl₂ in the case of sol H.

is shown under *c-d*. It should be ascribed to hydrogen ions displace the double layer into the intermicellary liquid. The amount of displaced hydrogen ions is not so prominent with Latekujan-F as it is with H.

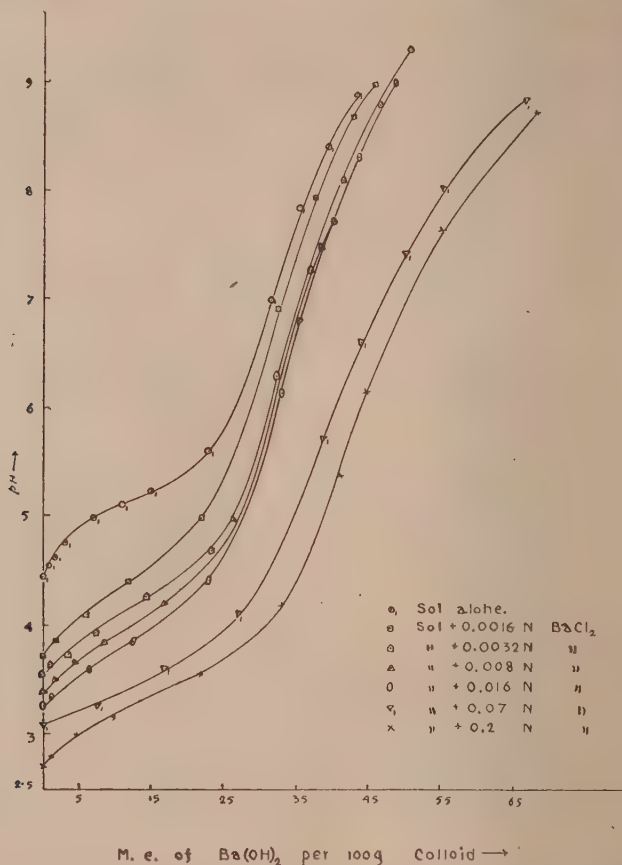
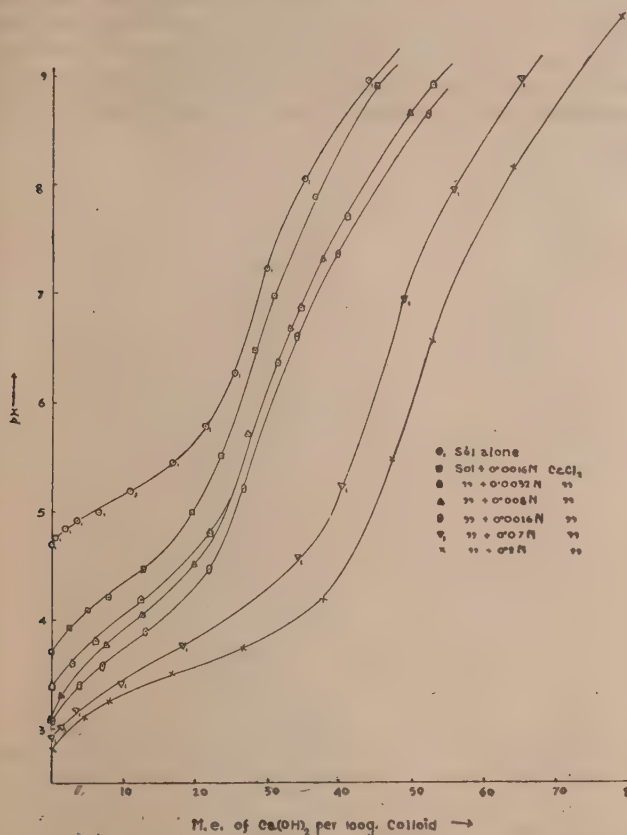


FIG. 2. Potentiometric titration curves with $\text{Ba}(\text{OH})_2$ of the sol Latekujan-F and salt mixtures

The variations in *c-d* with concentration of the added salt are regular with sol Latekujan-F. The errors involved in the estimation of *c* which are small quantities, especially at the lower concentrations of the salts, are magnified in the difference between them. The quantity *c* of Latekujan-F has, on the whole, a tendency to rise with increasing salt concentrations but it shows a constant decrease in the case of sol H. Unpublished work of Mitra from this laboratory also shows that the two sols have different electrochemical properties. This contrast is probably associated with the difference between the two soils from which the respective hydrogels have been prepared; sol H from neutral calcareous soil from Government Seed Farm, Kalyanpore (United Provinces) and sol Latekujan-

and acid soil on old alluvium from Government Farm at Latekujan (see Table I).

It is evident from the preceding and also from the work of Mukherjee [1942] that both H^+ and Al^{+++} ions are present in the intermicellar layer associated with the colloidal particles and both are displaced into the intermicellar liquid on the addition of neutral salts, but at higher concentration of the salt Al^{+++} ions form the major constituent.



3. Potentiometric titration curves with $Ca(OH)_2$ of the sol Latekujan-F and of its salt mixtures

The increase in the total acidity of a hydrogen clay sol on the addition of neutral salts indicates that more ions, Al^{+++} and/or H^+ ions, in addition to those already present in the interface are brought into a reactive condition. In order to ascertain to what extent these ions are displaced in the intermicellar liquid or remain associated with the colloidal particles the total acidities of the supernatant liquids of the 'sol and salt' mixtures and their supernatant liquids have been compared (Table I). The total reacting acids of the 'sol and salt' mixtures and of the supernatant liquid both increase progressively with the gradual

addition of a salt. The total acidity of the supernatant liquid, even for salt solutions is, however, less than that of the sol itself. Obviously active ions originally present on the surface are not displaced by the salt under these conditions. The larger total acidity of the 'sol and salt' mixture compared to that of the sol itself definitely shows, however, that active ions having a higher affinity for the surface have been rendered active and remain associated with the colloidal particles. When NaCl is the salt, the difference $a-b$, (Table I), which gives a measure of the additional acidity of ions rendered active, is greater than c , the total acidity of the supernatant liquid. In agreement with the weak displacing power of Na^+ ions, the number of ions rendered reactive is thus not completely displaced in the supernatant liquid. Consistent with the strong power of displacement of Ba^{++} ions, c is less than a .

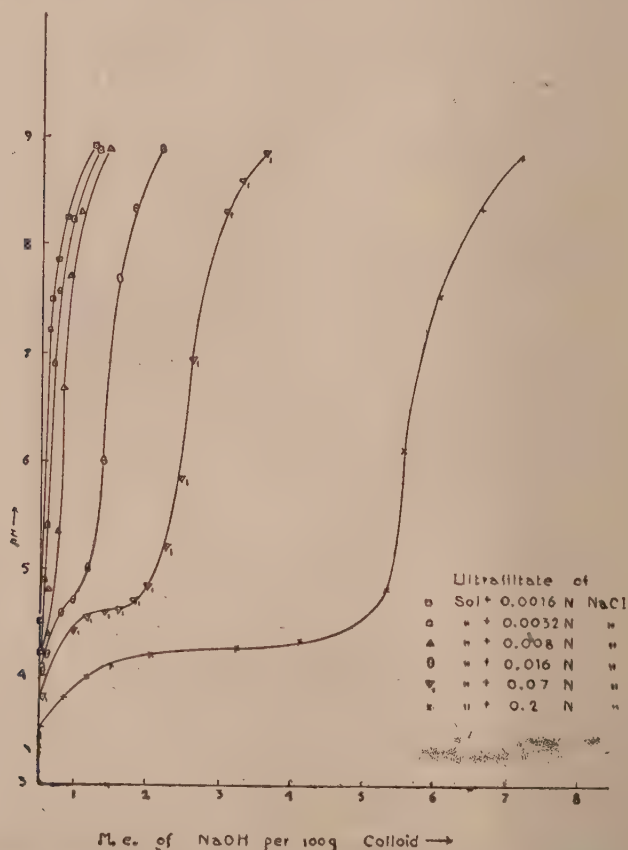
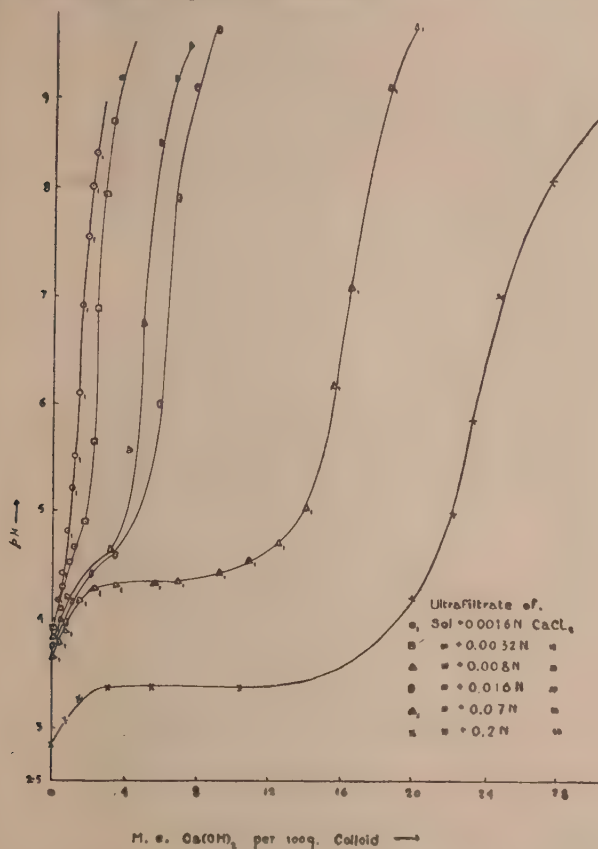


FIG. 4. Potentiometric titration curves with $\text{Na}(\text{OH})$ of the ultrafiltrates of Latekujan-F and NaCl mixtures

In order to ascertain the amount of these ions remaining associated with the surface in presence of salts the difference $a-c$, between the total acidity of the 'sol and salt' mixtures and that of the corresponding super-

d has been given in Table I. While $a-c$ is not constant, it does not differ greatly on the addition of salts or from the total acid of the sol. The quantity $a-c$ appears to depend on the displacing power of the cation of the salt and an equilibrium between the ions in the intermicellar space and in the double layer is indicated.



5. Potentiometric titration curves with $\text{Ca}(\text{OH})_2$ of the ultrafiltrates of the sol Latekujan-F and CaCl_2 mixtures

SUMMARY

Both H^+ and Al^{+++} ions are present on the surface of the colloidal particles of hydrogen clay, H^+ ions constituting a small fraction of the total. Of the total amount of these ions a portion is displaced into the intermicellar space on the addition of a neutral salt, while another portion remains associated with the colloidal particles. With increasing salt concentrations more and more ions (H^+ and Al^{+++}) are displaced into the supernatant liquid but the H^+ ions are brought into a reactive condition. The amount of ions remaining associated with the colloidal particles depends on the displacing power of the cation. A large reservoir of these ions on the surface is indicated.

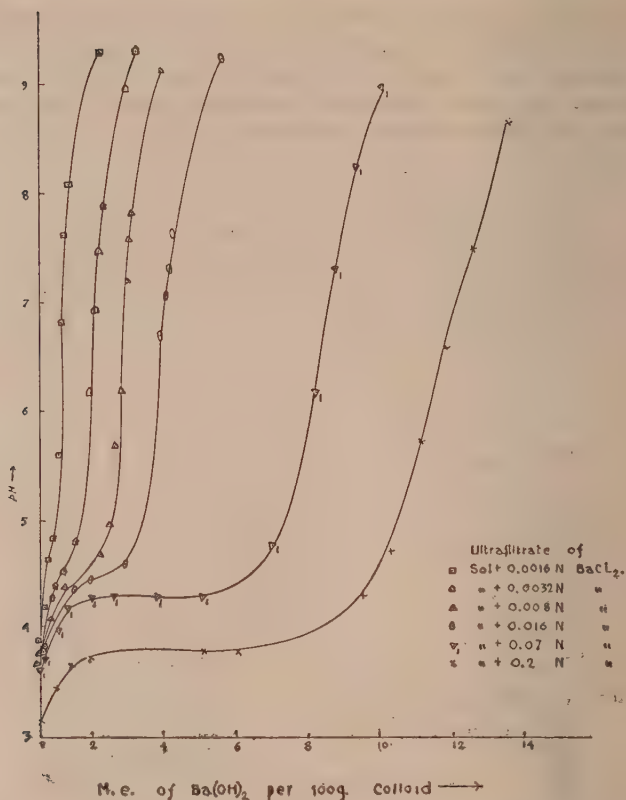


FIG. 6. Potentiometric titration curves with Ba(OH)₂ of the ultrafiltrates Lateknjan-F and BaCl₂ mixtures

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SOILS OF THE DECCAN CANALS

STUDIES IN AVAILABILITY OF NITROGEN IN SOIL WITH APPLICATION OF FARMYARD MANURE UNDER DIFFERENT CONDITIONS OF MOISTURE AND CARBON/NITROGEN RATIOS

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(With two text-figures)

THE application of farmyard manure in crop production is an essential item in Indian agriculture and is no exception under sugarcane growing in the canal zones of the Bombay-Deccan, where large quantities of the manure are frequently used for this crop. Yet, in spite of the long-established practice the use of farmyard manure in India, comparatively little scientific information is available regarding its efficacy in supplying available nitrogen to crops, or its ultimate effect in modifying soil properties, particularly the physical conditions.

In western countries the considerable amount of literature available on the subject has been reviewed by Jensen [1931]. Among recent work in India mention may be made of the investigation of Mukerji and Vishnoi [1936] on the rice soils of Raipur (Central Provinces). They have shown that the rate of decomposition of farmyard manure under submerged conditions approximates to that under aerobic condition and is higher in a medium clay than in a sandy loam soil. Mirchandani [1932] has stressed the importance of the C/N ratios in influencing the mineralization of farmyard manures in soils. Bal [1935], working on the rate of decomposition of added organic matter on the heavy black cotton soils of the Central Provinces, finds that the biological activities are at their best when the moisture content is about half the maximum water-holding capacity. Recently, Vishwanath [1937] has found heavy losses of nitrogen occurring under field conditions at Coimbatore during the nitrification of added ammonium sulphate, green manure and stable manure, the loss being greatest with ammonium sulphate. In spite of the heavy losses there was no movement of nitrogen into the deeper layers and there was no moisture saturation leading to denitrification. On the contrary, it has been observed in uncropped irrigated plots at the Sugarcane Research Station, Padegaon (unpublished data), that, with a heavy dressing of farmyard manure (60,000 lb. per acre), there was actually fixation of nitrogen to the extent of 74 per cent over the original within six months. But where there was very little nitrification, the nitrate levels of the manured plots

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being not appreciably higher than those of the control during this period of experimentation. Similarly, from replicated experiments with cane conducted at the same station for three years, there is reason to believe that the contribution of farmyard manure to nitrogen nutrition of the crop is negligible, whereas its beneficial effect in creating a desirable soil-tilth is quite marked in the case of a shallow-rooted cane variety [Rege, 1941].

The most important aspect of this question, however, is that, in cane farming, where it has been the practice to add heavy dressings of farmyard manure, there would not only be an enormous waste of the manure if indiscriminately used, but also, according to indications obtained in the course of a fertility survey of cane soils in the Deccan, the ultimate effect of such a practice would lead to soil deterioration by the widening of the C/N ratio under such conditions. The results of the above-mentioned survey showed that cane soils showing signs of deterioration, i.e. where more nitrogen is required every year to produce the same yields, had in the majority of cases higher C/N ratios than the normal fertile soils. It was, therefore, felt that the solution of this problem would have an important bearing on the economics of cane growing and on the equally important question of the maintenance of soil fertility.

Accordingly, pot-culture experiments were carried out during the period 1935-37 to investigate fully the availability of the manure in different soils and its ultimate effect on their fertility status. In the present paper the question will be dealt with from the points of view of moisture condition and C/N ratios in one of the soil types which occurs at the farm.

EXPERIMENTAL

Soils and manures used

The soils used in these experiments belong to the group of typical Deccan cotton soils which overlie the Deccan Trap—a volcanic formation of basaltic rocks. Recently they have been classified according to the modern system [Basu and Sirur, 1938] and the present work deals with the decomposition of manures in one of the important soil types—called the 'B' type—of which a brief description of which is given below :—

The profile

Horizons	Description
I	Uniform dark grey with a brown shade, interspersed with roots, loam :— (a) Large clods, 2-3 in. in diameter. (b) Smaller clods $\frac{1}{2}$ -1 in. in diameter, more friable than above.
II	Mottled horizon, brown intermingled with greyish black, brown dominating in lower layers, silt loam.
III	Reddish brown colour, with white concretions of lime and siliceous material—more compact than above—clay.
Below	Hard murrum (decomposed trap).

The depth of soil above the murrum varies from about 3 to 12 ft.

Soil characteristics

In the present work only the first foot of soil has been used for the experiment. Some important characteristics of this depth are given in Table

TABLE I

General characteristics of the soil

(A) Bulk chemical analysis

Residue insoluble in HCl (per cent)	Soluble SiO ₂ (per cent)	Fe ₂ O ₃ (per cent)	Al ₂ O ₃ (per cent)	CaO (per cent)	MgO (per cent)	K ₂ O (per cent)	Na ₂ O (per cent)	P ₂ O ₅ (per cent)
30.29	14.74	10.40	14.55	7.14	1.48	0.110	0.957	0.075

(B) Other property

Mechanical analysis		Exchangeable bases m. e. per cent				pH value		Calcium carbonate per cent
Clay per cent	Silt per cent	Ca	Mg	K	Na	in water	in N KCl	
61.75	14.75	44.25	10.87	3.96	4.10	8.42	7.51	9.01

The general nature of the locally available farmyard manures will be seen from Table II where analytical data for seven typical manures and one sample of compost (i.e. No. 7) prepared at the Padegaon Farm are given. The farmyard manures are usually prepared from the waste materials of *jowari* (*Andropogon sorghum*) straw left after feeding the cattle, together with their urine and dung, while compost has been prepared out of sugarcane trash.

TABLE II

General analyses of farmyard manures and compost

Sample No.	Age of manure in months	Loss on ignition	Carbon	Nitrogen	Humus	Per cent humified matter	C/N ratio	pH in water
		Per cent on air-dry basis						
1	2	3	4	5	6	7	8	9
1	6	29.2	12.15	0.847	6.15	29.4	14.3	7.37
2	12	21.7	8.61	0.789	7.25	48.9	11.7	7.45
3	12	19.7	5.85	0.582	3.43	34.0	10.1	8.12
4	10	26.1	9.98	0.946	5.72	33.2	10.6	7.45
5	9	21.3	6.06	0.606	4.91	47.0	10.0	7.95
6	8	27.5	7.04	0.727	8.63	71.1	9.7	7.12
7	8	35.1	7.53	0.811	6.98	53.8	9.3	7.20
8	24	11.2	5.17	0.483	4.77	53.5	10.7	7.20

It will be noticed that the C/N ratios are usually round about 10—unless the sample is taken too raw as in No. 1—and the nitrogen content varies from 0.48 to 0.95 per cent. The per cent humified matter varies from 29.4 to 71.1 and does not seem to bear any well-defined relation with the age of the manure.

Technique followed

The decomposition studies described in this paper were conducted under conditions corresponding to those under alternate wetting and drying of soil as occurs under sugarcane cultivation in the Deccan. Fifteen kilograms of air-dried soil were used in this experiment after thorough mixing of required quantities of manures and distilled water. The treated soils were placed in glazed earthenware pots (1 ft. diameter \times 1 ft. height) and packed so as to occupy a constant volume in each pot. The pots were then exposed to the atmospheric conditions in a room. The pots were weighed at regular intervals to find out the loss by evaporation. Representative samples for the various determinations were taken after thorough mixing of the soil. The required quantity of water was added to the soils with proper stirring and the packing adjusted uniformly in all the pots. Care was taken to keep the time of sampling, water addition and plating for bacterial work constant throughout the experiment. Ammoniacal nitrogen, nitrate nitrogen, bacterial number and studies in respiration were done on the fresh samples, the results being calculated on oven-dry soil by keeping a separate sample for moisture determination. Carbon and nitrogen were determined on oven-dried samples and figures are reported on oven-dry basis.

The following analytical methods were employed :—

Ammoniacal nitrogen was determined by extracting the soil by 2N NaOH at pH 1.0 as recommended by C. Olsen [Wright, 1934].

Nitrate nitrogen was determined by the phenol-disulphonic acid method recommended by A. O. A. C. [Methods of Analysis, A. O. A. C., 1930].

Total nitrogen was determined by the routine Kjeldahl method using the modification of Bal [1925].

Carbon was determined by the wet combustion method [Leather, 1925]. It was found, however, that destruction of carbonates was not complete within the period of $\frac{1}{2}$ hour using water bath. After a number of trials with the best cotton soils of the tract, which are usually highly clayey and calcareous, a prolonged period of heating for two hours was found necessary, however, with the precaution of having a Liebig's condenser attached to the flask throughout the heating in order to avoid excessive concentration of the mixture. Also, later, during the heating of the soil with potassium dichromate, an oil bath at a temperature of 130°C. was resorted to, the aspiration being continued for six instead of five hours.

Humus was determined by Sigmond's method, by extraction with 10% sodium carbonate [Sigmond, 1927].

Numbers of bacteria were determined by the plate method, using an agar medium of Thornton [1922] containing K_2HPO_4 1 gm., $MgSO_4 \cdot 7H_2O$ 1 gm., $CaCl_2$ 0.1 gm., NaCl 0.1 gm., KNO_3 0.5 gm., $FeCl_3$ 0.002 gm., asparagine 0.5 gm., mannitol 1 gm., agar 20 gm., water to 1000 c.c. The quantity of agar had to be increased to 20 gm. from the recommended 15 gm. as otherwise the medium was not solidifying under the climatic condition here; it was adjusted in each case to 7.4 as recommended.

Ten gm. of the fresh soil sample were shaken up for four minutes with 250 c.c. of a sterile saline of 0.5 per cent NaCl and 0.05 per cent $MgSO_4$ (solution a). Subsequent dilutions corresponding to 1/2500 (dilution b)

150,000 (dilution *c*) were prepared from suspension (*a*) and (*b*), respectively, adding requisite quantities of sterile saline. Throughout the experiment, suspensions (*b*) and (*c*) were used for plating, taking 1 c.c. each of the above suspensions in sterile petri dishes and adding to them 10 c.c. lots of sterile medium. The petri dishes were incubated for 5 days (which was found to be the optimum period) at a temperature of 35°C. Five replicates were kept in each case, the average figure being taken for calculation of the bacterial number. The standard error was found to be within 20 per cent in the majority of cases.

Evolution of CO₂.—From the periodical soil samples collected from the plots, 250 gm. were taken in conical flasks and the CO₂-evolution measured after every 24 hours by absorption in standard baryta solution in Pettenkofer's tubes by aspiration of a steady stream of CO₂-free air through the apparatus. The CO₂-evolution was followed up for a period of nine days during which it came down to a constant and negligible level. The CO₂-evolution during this nine-day period was then divided by 9 to get the daily average, and these figures were entered against the day of sampling for comparison.

DATA AND DISCUSSION

First series of experiments

In this experiment the decomposition in soil of a sample of farmyard manure (No. 2 of Table II) was studied with the moisture contents of soil at field saturation and at half saturation, the moisture being made up at weekly intervals to 44 per cent and 22 per cent respectively. These moisture levels were found to represent, more or less, the average conditions of moisture obtaining under periodical heavy irrigation usually given to cane, and under the normal rainfall in the tract, respectively. The soil used in this experiment was that of a normal fertile soil obtained from an experimental block on the Adegaon Farm and had a carbon/nitrogen ratio of 14.9 at the time of the experiment.

The treatments were as follows :—

I. Control soil—no manure.

*II. Addition of farmyard manure corresponding to 0.33 per cent of the soil used.

*III. Addition of farmyard manure corresponding to 1 per cent of the soil used.

Periodical determinations of bacterial numbers, ammonia and nitrate were conducted every week till 42 days, whereas carbon and total nitrogen were determined once at the start and again after 200 days in order to allow sufficient time for soil changes to take place.

Nitrogen changes and bacterial numbers

The ammoniacal and nitrate-nitrogen figures for different periods are given in Tables III and IV.

* These applications of manure work up to 10,000 lb. and 30,000 lb. per acre respectively under field conditions.

TABLE III

Ammoniacal nitrogen in mg. per 100 gm. of air-dry soil under field and half moisture saturations in control and farmyard manure treated pots

(Normal fertile soil)

Treatment*		Number of days from commencement						
		0	7	14	21	28	35	42
I. No manure	F.	1.22	1.55	0.60	0.60	2.69	3.56	1.20
	H.	1.22	0.53	0.71	0.73	0.57	0.63	0.52
II. Farmyard manure (0.33 per cent)	F.	1.22	1.54	0.81	0.76	2.84	1.20	1.08
	H.	1.22	1.03	0.71	0.60	0.43	0.68	0.37
III. Farmyard manure (1 per cent)	F.	1.22	1.55	1.25	0.76	3.00	1.17	1.35
	H.	1.22	1.06	0.76	1.02	0.89	0.57	0.41

*F = Field moisture saturation ; H = Half moisture saturation

Generally speaking, there is more accumulation of ammoniacal nitrogen at field-moisture saturation than at half saturation both in control and manured soils. The levels of ammonia are not much affected by the addition of farmyard manure excepting on one occasion (i.e. 35th day) when considerable lowering in ammonia is observed at field-moisture saturation.

TABLE IV

Nitrate nitrogen in mg. per 100 gm. of air-dry soil under field and half moisture saturations in control and farmyard manure treated pots

(Normal fertile soil)

Treatment		Number of days from commencement						
		0	7	14	21	28	35	42
I. No manure	F.	0.16	0.15	1.20	0.94	1.06	0.61	0.86
	H.	0.16	0.10	0.08	0.07	0.10	0.14	0.27
II. Farmyard manure (0.33 per cent)	F.	0.16	0.23	0.16	0.21	0.63	0.92	0.82
	H.	0.16	0.26	0.31	0.22	0.30	0.36	1.93
III. Farmyard manure (1 per cent)	F.	0.16	0.17	0.18	0.23	0.20	1.76	0.96
	H.	0.16	0.51	0.60	0.32	0.35	0.32	2.52

The nitrate contents of soils are also maintained at a higher level at field-moisture saturation than at half saturation but, while farmyard manure generally lowers the nitrate at the former moisture level, it raises the value especially on the 42nd day, in both doses of farmyard manure at the latter moisture level.

The relationship between the bacterial number and mineral nitrogen (i.e. ammonia plus nitrate) is given in Fig. 1.

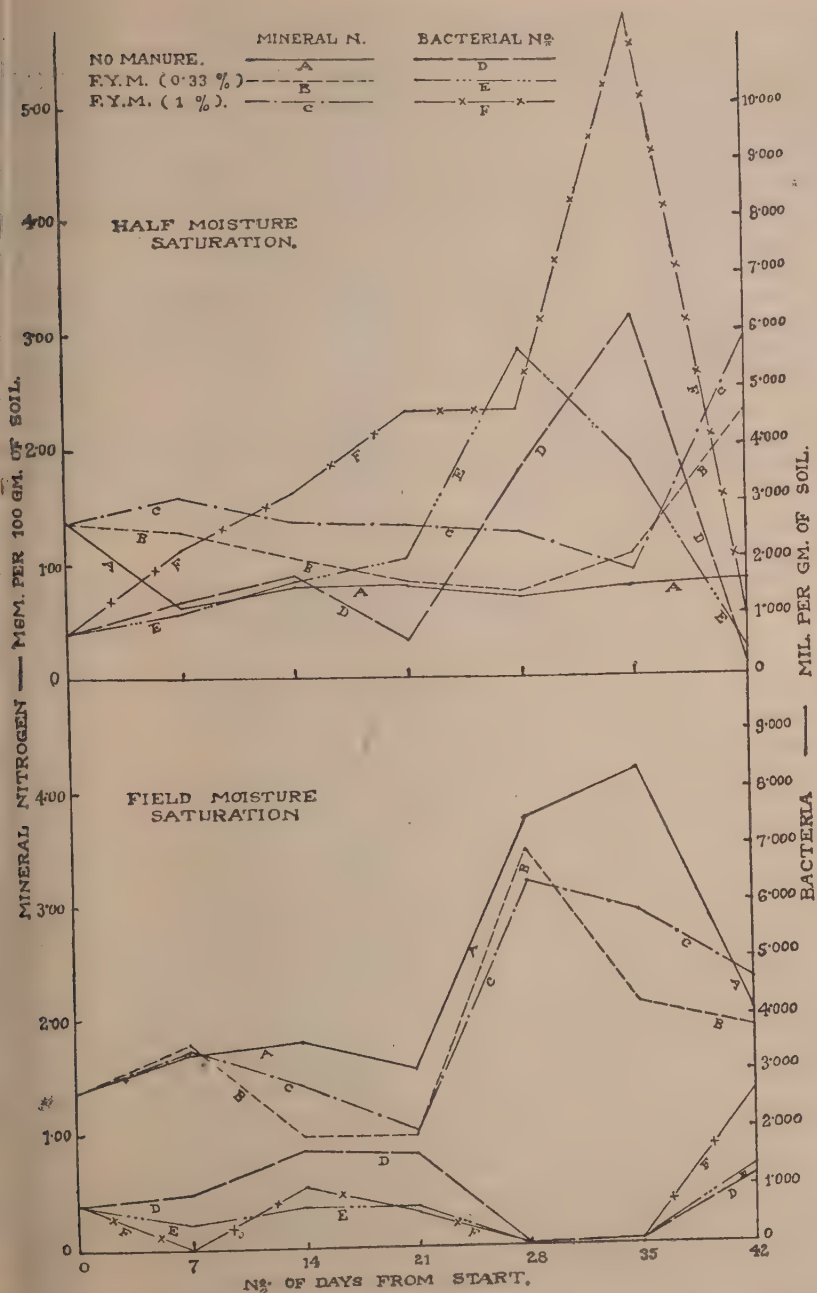


Fig. 1. Periodic fluctuations in mineral nitrogen and bacterial numbers—normal fertile soil

Although mineral nitrogen shows generally a higher level at field saturation than at half-saturation moisture in all the treatments, the effect of addition of manure is quite different at two moisture levels. Thus while farmyard manuring has helped to raise the mineral nitrogen status of soil at half-saturation it has affected it adversely at field saturation. This fact is of considerable importance in the economy of sugarcane farming on the Deccan Canals where addition of large amounts of bulky manures is usually practised by the cane cultivators, resulting in their great wastes. This non-availability of nitrogen in farmyard manures on 'B' type* of soil has been actually supported by experiments conducted at the Padegaon Farm [Rege, 1941].

Referring to Fig. 1, it will be observed that the bacterial activity as measured by plate counts indicates a general lowering in the activity on addition of manure at field-moisture saturation, whereas increased activity is shown by this treatment at half-moisture saturation. This clearly reflects the unfavourable conditions created for bacterial growth by manure at field moisture. Similar depressing effects of manures on bacterial numbers were also found by Mukerji and Vishnoi [1936] while working on the heavy soils of Raipur, Central Provinces. They have attributed it to the formation of some toxic product which proves harmful to the bacteria capable of growth on Thornton's agar.

One interesting thing to be observed in this connection is the fact that in spite of lower bacterial numbers shown at field moisture the mineralization of nitrogen is quite high when compared with figures at the half-saturation moisture. Apparently the more useful types of organisms which are responsible for mineralization of nitrogen must be present in larger numbers (or in more reactive form) at field moisture but which are not reflected in mere plate counts.

Now, coming to the relationship between the fluctuations in the mineral nitrogen contents at different periods and bacterial numbers, two things must be borne in mind, viz. (a) the mineral nitrogen present at any moment in the soil is the balance between that which is produced by micro-organisms minus the amount taken up by microbial cells plus the nitrogen lost by denitrification and loss in gaseous forms; (b) the bacterial counts by Thornton's plate method do not include all the micro-organisms, especially the cellulose-decomposing bacteria, and thus any relationship observed between mineral nitrogen and bacterial number must be of a qualitative nature. With these comments we find a fair amount of negative correlations between mineral nitrogen and bacterial numbers. At field-moisture saturation the bacterial numbers generally rise till 21st day after which there is a fall on the 28th, 35th day and a rise again. The mineral nitrogen also shoots up on the 21st and 35th day when the bacterial numbers are very low and falls again when the numbers rise. At half-moisture saturation the bacterial numbers rise till 28th or 35th day and then attain very low values on the 42nd day. The mineral nitrogen which steadily falls with rise in bacterial number, rises again only when the number goes down very low. This sort of inverse relationship between bacterial number and mineral nitrogen has also been observed

* The availability of nitrogen in farmyard manure in other soil types was discussed in a subsequent paper.

[1931] at Rothamstead, who attributed the lowering of mineral nitrogen absorption by the bacterial cells and its release on the death of the bacterial bodies resulting in higher production of mineral nitrogen in the soil. It would be thus evident that in the different treatments, the greater bacterial number prior to its attaining the lowest values, the greater will be the release of mineral nitrogen at the cessation of the bacterial activity. The contrary behaviour of farmyard manuring on the production of mineral nitrogen (at the cessation of bacterial activity) in the two moisture levels are clear.

changes in soil under different treatments

The changes in carbon, nitrogen and C/N ratios in soils were determined after 200 days in different treatments and are shown in Table V. The figures in brackets indicate the calculated values taking the original values as 100.

TABLE V

changes in carbon, nitrogen and C/N ratios in differently treated soil after 200 days

Treatments	Nitrogen per cent			Carbon per cent			C/N ratio per cent		
	Original	Final		Original	Final		Original	Final	
		F	H		F	H		F	H
Control	0.049 (100)	0.050 (102.04)	0.049 (100)	0.73 (100)	0.67 (91.78)	0.71 (97.26)	14.9 (100)	13.4 (89.93)	14.5 (97.32)
Farmyard manure (per cent)	0.051 (100)	0.063 (123.53)	0.058 (113.73)	0.74 (100)	0.99 (133.78)	0.80 (108.11)	14.5 (100)	15.7 (108.28)	13.8 (95.17)
Farmyard manure (1 per cent)	0.054 (100)	0.068 (125.93)	0.058 (107.41)	0.78 (100)	1.15 (147.44)	0.84 (107.69)	14.4 (100)	16.9 (117.36)	14.5 (100.69)

It will be seen that the nitrogen contents of the control soil remain practically unchanged even after 200 days under both the moisture conditions. With the addition of farmyard manure (treatments II and III), however, there have been increases in nitrogen with both the doses of manure. Since free-living nitrogen-fixing organisms require energy-materials [Waksman, 1931], the addition of farmyard manure is beneficial as it supplies all the necessary energy while undergoing oxidation in the soil. In the present experiment it was noticed that there is more gain in nitrogen at field saturation than at half-saturation moisture which suggests the possibility of anaerobic bacteria like *Clostridium pasteurianum* taking an active part in these soils.

With regard to carbon there is only a slight lowering in the values in the control soil, whereas in all the other treatments there is a gain. Increase in carbon is more pronounced at field saturation than at half-saturation.

With the addition of farmyard manure, the increases are 34.47 per cent at field-saturation for the lower and higher doses of manuring respectively, while in the case of half saturation the increases are much smaller. The question of such increases in the carbon contents of soil due to manuring requires further investigation in a separate experiment with different manure materials. This is described later.

Finally, as a result of lowering in carbon in the control soil, the C/N decreases a little, especially at field-moisture saturation. In the case of yard manuring, although there are increases in both carbon and nitrogen are almost balanced in the case of half-saturation moisture but at field-moisture saturation there is an ultimate increase in the ratio in both the treatments.

Second series of experiments

With regard to the fixation of carbon that was found to take place in the previous experiment, a separate investigation was conducted with different samples of manure on a second sample of farm soil having a C/N ratio of 14.35. The experiment was carried out in flat glass dishes with 200 g of soil with addition of 2 gm. of manure at 44 and 22 per cent moisture respectively, for a period of one month. The soils were not stirred in the experiment and exposed to sunlight every day for one hour in order to encourage the algal growth if any. The results are given in Table VI.

TABLE VI

Changes in carbon, nitrogen and C/N ratios in soil with application of different samples of farmyard manure at two moisture levels†

Period	Farmyard manure No. 1* (6 months' old)						Farmyard manure No. 2* (12 months' old)					
	F			H			F			H		
	Carbon	Nitrogen	C/N	Carbon	Nitrogen	C/N	Carbon	Nitrogen	C/N	Carbon	Nitrogen	C/N
Original.	0.84 (100)	0.058 (100)	14.5	0.84 (100)	0.058 (100)	14.5	0.80 (100)	0.057 (100)	14.2	0.80 (100)	0.057 (100)	14.2
A month after	0.88 (104.76)	0.064 (110.34)	13.62	0.84 (100)	0.062 (106.90)	13.5	0.87 (108.75)	0.056 (98.25)	15.4	0.82 (102.50)	0.066 (115.7)	12.5

Period	Compost No. 7* (8—10 months' old)						Farmyard manure No. 8* (2 years' old)					
	F			H			F			H		
	Carbon	Nitrogen	C/N	Carbon	Nitrogen	C/N	Carbon	Nitrogen	C/N	Carbon	Nitrogen	C/N
Original.	0.79 (100)	0.057 (100)	13.7	0.79 (100)	0.057 (100)	13.7	0.77 (100)	0.054 (100)	14.2	0.77 (100)	0.057 (100)	12.8
A month after.	0.80 (101.27)	0.063 (110.53)	12.7	0.76 (96.20)	0.065 (114.04)	11.7	1.02 (132.47)	0.063 (116.67)	16.10	0.81 (105.19)	0.057 (109.2)	14.7

* These numbers refer to those given in Table II

† F = Field-moisture saturation; H = Half-moisture saturation

It will be observed that the C/N ratios have gone down to a certain extent in all treatments with the exceptions of farmyard manures Nos. 2 and 8 where there are slight increases in the values at field-moisture saturation. Looking into the individual figures of carbon and nitrogen it will be noticed that in the case of soil treated with a fresh manure (No. 1, i.e. 6 months old) there is practically no increase of carbon but the gains in nitrogen are 10 and 7 per cent respectively at field and half-moisture saturation.

and half saturation respectively. With manure No. 2 (1 year old, which was used also in the previous experiments) increase in carbon to about 9 per cent is noticed in field-saturation moisture and a gain in nitrogen of 16 per cent is noticed at half-saturation. Using compost prepared at the farm there is practically no increase in carbon under both the moisture conditions, whereas gains in nitrogen from 11 to 14 per cent are observed. In the case of soil treated with manure No. 8 (2 years old) there have been 32 per cent and 6 per cent increases in carbon in field and half saturation respectively, whereas gains in nitrogen are 17 and 9 per cent. This experiment shows definitely the varying nature of manure samples with regard to inducing fixation of carbon and nitrogen. As soil-algae are known to be capable of synthesizing complex organic substances from CO_2 and water in sunlight [Russell, 1923], it is only natural to attribute this increase in carbon to this cause. Further, recent experiments in the laboratory have demonstrated considerable algal growth in soils treated only with manure Nos. 2 and 8 at field-moisture saturation.

Third series of experiments

Two soils were chosen for this experiment, having fairly high C/N ratios; the carbon content of one was about three times that of the other. The object of the experiment was to test whether, apart from the question of high C/N ratio, the actual amounts of carbon and nitrogen affect the decomposition of manures in any way. Both these soils had the same history, namely, that wheat was grown on them for a long time and the yield was falling off gradually during the past years. Both belonged to the same soil type (i.e. B type). One of the soils had a ratio of 22.5 (possessing both higher carbon and nitrogen contents) while the other had a ratio of 17.3, having lower carbon and nitrogen contents. (It is rather unfortunate that exactly the same ratio was not obtainable in these two soils having the same past history and belonging to the same soil type). The treatments were the same in both the soils, viz.—

I. Control soil—no manure.

II. Addition of farmyard manure* corresponding to 1 per cent of soil used.

Soils were kept at half-moisture saturation by addition of water every 9th day when soil samples were also given a thorough stirring by hand. Detailed determinations were continued up to a period of 81 days. In the discussion that follows the soil having higher contents of both carbon and nitrogen will be referred to as No. 1 and the other as No. 2.

Nitrogen changes and bacterial numbers

In Table VII, the mineral nitrogen contents at different periods for the two soils are given.

It will be observed that the mineral nitrogen contents of soil No. 1 are generally higher than those of soil No. 2 in the case of the untreated soils. The application of farmyard manure has not resulted in any increase in mineral nitrogen in the case of soil No. 1. On the contrary a slight lowering in the values is observed at the earlier stages. With soil No. 2, farmyard manure has given some additional mineral nitrogen over the control. These results

* In this series the sample of farmyard manure used is the same as No. 2 of Table II

will be quite clear from Table VIII where the per cent mineralization figures for different periods are given. For the sake of comparison similar figures for the normal fertile soil already described under the first series of experiments are included.

TABLE VII

Mineral nitrogen in mg. per 100 gm. of air-dry soil at half-moisture saturation for soil Nos. 1 and 2 with and without farmyard manure deteriorated soil

Serial No.	Treatment	Days								
		0	9	18	27	36	45	54	63	72
1	No manure	1.46	1.82	1.16	1.49	1.74	1.37	1.21	1.27	0.98
	Farmyard manure (1 per cent)	1.46	0.78	0.84	1.27	1.56	...	1.41	1.31	1.23
2	No manure	1.12	1.14	0.72	1.53	0.97	0.85	0.86	1.02	0.90
	Farmyard manure (1 per cent) .	1.12	1.08	1.26	1.60	1.22	1.81	1.03	1.19	1.16

TABLE VIII

Periodic excess of mineral nitrogen over the control expressed as percentages added nitrogen in the form of farmyard manure to fertile and deteriorated soils at half-moisture saturation

	Number of days from commencement					
	0	7	14	21	28	35
Normal fertile soil C/N = 14.9	0.00	12.72	7.71	7.31	7.71	1.62

	Number of days from commencement								
	0	9	18	27	36	45	54	63	72
Soil No. 1 C/N = 22.5	0.00	-14.07	-4.33	-2.98	-2.44	...	2.71	0.54	3.38
Soil No. 2 C/N = 17.3	0.00	-0.81	7.37	0.95	3.38	12.99	2.30	2.30	3.52

The poor responses of the farmyard manure in the deteriorated soils possessing higher C/N ratios are at once self-evident. Soil No. 1 in this respect is very much inferior to soil No. 2.

Referring now to Fig. 2 the bacterial numbers show very small fluctuations in the case of both the soils except that at earlier stages soil No. 1 exhibits much lower numbers. Generally speaking, farmyard manuring enhanced the bacterial activity in both the soils as was also observed in the case of normal fertile soil at half-moisture saturation. The differences in the periodic fluctuations in the bacterial numbers and mineral nitrogen in the deteriorated and normal soils are worth observing (Fig. 1). The sudden release of mineral nitrogen is not observed in the case of the deteriorated soils where

rial numbers attain low figures as in the case of the normal soil. This
 iar behaviour of the deteriorated soils requires further careful studies.

	MINERAL N.	BAC. N ^o .	CO ₂ EVOLUTION.
SOIL ALONE	A	C	E
SOIL + F.Y.M. (1%)	B	D	F

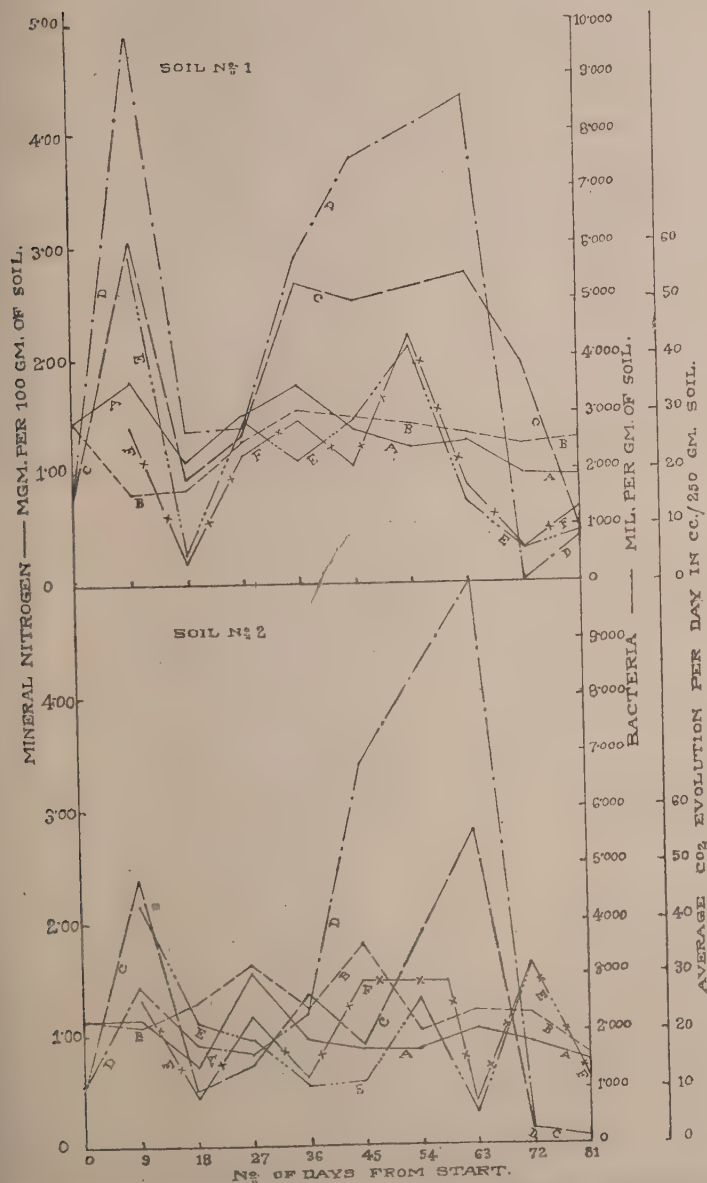


Fig. 2. Periodic fluctuations in mineral nitrogen, bacterial numbers and carbon dioxide evolution—deteriorated soils

Respiration studies

Respiration studies were conducted on these two soils and the results also shown in Fig. 2. The evolution of carbon dioxide from the two soils indicates, on the whole, better microbiological activities in soil No. 1 than in soil No. 2, which fact is also borne out by bacterial counts. It was apparent that, in general, the periodicity of CO_2 -evolution shows similar trends to bacterial activity in both the soils. The addition of farmyard manure has, however, shown an initial set-back in figures of CO_2 in both soils, while in soil No. 1 the average production is also lowered by manuring. It is, however, difficult to reconcile these opposite facts (i.e. increased bacterial activity and lowering in CO_2 -evolution by farmyard manuring) unless fixation of algae within the short period between stirring of the soils with the subsequent absorption of CO_2 is assumed.

Changes in the soils after 327 days of alternate drying and wetting at half-moisture saturation

The process of wetting (to 50 per cent of the moisture-holding capacity) and drying of the soils in pots with regular periodic stirring was continued till 327 days to see the ultimate effect on the soil. The soil samples after this period were air-dried and total nitrogen and carbon determined. The results calculated on 100 gm. of oven-dry soil are given in Table IX, together with the C/N ratios and the per cent increases over the original in brackets.

TABLE IX

Final changes in nitrogen, carbon and C/N ratios in differently treated soils after 327 days

Soil No.	Treatment	Control			Farmyard manure (1 per cent)		
		N	C	C/N	N	C	C/N
1	Original	0.067 (100)	1.51 (100)	22.5 (100)	0.076 (100)	1.64 (100)	21.6 (100)
	Final	0.086 (128.36)	0.99 (65.56)	11.5 (51.11)	0.101 (132.89)	1.10 (67.07)	10.9 (48.18)
2	Original	0.030 (100)	0.52 (100)	17.3 (100)	0.040 (100)	0.65 (100)	16.3 (100)
	Final	0.049 (163.3)	0.66 (126.8)	13.4 (80.8)	0.060 (150.0)	0.52 (80.00)	8.3 (50.9)

Table IX shows that there are increases in nitrogen even in the control soil, the increase being more in soil No. 2 where the nitrogen content at start was very low. It appears that, unlike the normal soil these soils with high C/N ratios are capable of fixing nitrogen even without addition of energy material from outside sources. With regard to carbon there is a slight gain in the case of soil No. 1 but in the case of soil No. 2 (where the original carbon was low) there is a slight gain. The ultimate result is, however, a lowering in the C/N ratio in both the soils. (This is more pronounced in soil No. 1 than in soil No. 2). With the addition of farmyard manure (energy material) there is fixation of nitrogen in both cases although the percentage increase

not very much different from those observed in the control soil. Losses of carbon occur in both the soils and the resulting C/N ratios are much lower than the original values. Addition of farmyard manure appears to be more effective in lowering the ratio in the case of soil No. 2. The above experiment tests the possibility of reclaiming soils having high C/N ratio by the simple process of wetting and drying with regular bulking of the soil (i.e. light cultivation operations) which may be further helped by the addition of farmyard manure in case the soil is poor in both carbon and nitrogen.

SUMMARY AND CONCLUSIONS

With reference to the poor response of sugarcane to application of farmyard manure which was observed in the experiments conducted at the Pade Farm, and also, in order to investigate the important question of the rôle of bulky manures in modifying soil fertility under continued cane growing, a series of experiments were carried out on an important genetic soil type belonging to the broad group of black cotton soils. The results are given below:

In the first series the biological responses were studied of a normal fertile soil obtained from an experimental block on the farm to the application of two doses of farmyard manure. Moisture was maintained at two levels in two different sets of pots, one at field saturation and the other at half saturation, approximating to conditions under perennial irrigation and dry farming, respectively.

In general, the mineral nitrogen contents of soils at field-moisture saturation was higher than those at half-moisture saturation. The application of farmyard manure, however, raised the mineral nitrogen status at half-moisture saturation, while it adversely affected the status at field saturation. Serial counts taken periodically showed lowered bacterial population as a result of manuring at field-moisture saturation, while reverse was the case at half-moisture saturation. Deleterious effect of field-moisture saturation on the mineralization of farmyard manure is thus indicated.

The changes in carbon and nitrogen status of soils after 200 days were interesting. Although no appreciable changes were noticed in the untreated soils, considerable increases, both in carbon and nitrogen, were observed at field-moisture with the addition of farmyard manure, and the resulting C/N ratios were raised in both the doses of manuring. In the case of half-moisture saturation, though slight increases in these constituents were noticed, the ratios remained practically unaffected.

In order to find out whether this increase in carbon due to farmyard manure application is a general one, a second series of experiments was conducted with four samples of farmyard manure at two moisture levels without disturbing the soils. The results indicated the specific nature of manures in affecting fixation of carbon in soils under such conditions within a short period. Further experiments have demonstrated that the presence of algae in certain manure samples is responsible for this fixation by their development at field-moisture saturation. Thus, samples of manure which were found to contain no algae, were unable to increase the carbon contents of soils.

In a third series of experiments, two samples of soil where cane yields were low and which showed high C/N ratios were taken for study. The

availability of nitrogen by the application of farmyard manure half-saturation moisture was found to be very poor in these soils compared to a normal fertile soil having a lower C/N ratio. In spite of bacterial activity as a result of farmyard manuring in these soils, mineralization of nitrogen was found to be defective. This defective mineralization of nitrogen in these soils requires further careful microbiological studies.

The final changes in the carbon and nitrogen status of these soils make it possible to consider the possibility of ameliorating these soils (by lowering the C/N ratio) by repeated wetting and drying in conjunction with regular bulking of the soil periodically.

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STUDIES ON INDIAN RED SOILS

FACTORS RESPONSIBLE FOR BUFFER CAPACITIES AND BASE-EXCHANGE PROPERTIES

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(With three text-figures)

LL soils possess considerable but widely different buffer capacities, which means that these substances contain active material which tends to interact the changes in the reaction brought about by the addition of basic or acidic substances. Besides, small amounts of dissolved buffering salts such as phosphates, carbonates and salts of organic acids, both clay and humus, act as strong buffers.

Raychaudhuri and Nandymazumdar [1940] have shown that buffer curves of profile samples of Indian red soils indicate a more or less definite inflexion at pH 9.8 and frequently a second inflexion either at pH 2.9 or 3.6. It was felt desirable to examine closely the factors which are responsible for the inflexion of the buffer curves at these pH values. Profile samples of red soils of India, collected from Bihar, Orissa, Assam and parts of Bengal have been used in this work. The chief base-exchange properties of these soils, including the pH , air-dry moisture, total exchangeable bases, degree of base saturation, buffer curves and other related properties were determined. For the determination of buffer curves the method devised by Schofield [1933], has been followed. It has been pointed out by Raychaudhuri [1941] that the study of buffer curves by Schofield's method yields very valuable information regarding the nature of soil types.

EXPERIMENTAL

Determination of pH

The pH values at soil-water ratio 1 : 2.5 were determined by Kuhn's barium sulphate method using a Hellige colorimeter and also by the quinhydrone electrode method.

Determination of carbonates

The determination of carbonates were made with the help of Collin's gas buret.

Determinations of air-dry moisture

The air-dry moisture was calculated from the loss in weight of 2—5 gm. of soil heated in an electric oven at a temperature of 105°C. for 24 hours.

Determination of buffer curves

A weighed quantity of soil was taken in a 40-c.c. dried test tube fitted an indiarubber stopper and 25 c.c. of the buffer solution were added. rubber stopper was fitted and the test tube was clamped to a rotating sh which was rotated with the help of an electric motor for a period of 16 h The test tubes were taken out of the shaker and allowed to stand for nearly hours. A measured volume of the supernatant liquid was pipetted off titrated with a standard HCl or lime solution, as the case may be. the case of buffer solutions of pH 1.3, 2.9, and 4.6, the figures for the up were corrected for the percentage carbonate present in the soil wherever soil contained measurable amounts of carbonate. 0.06 N organic (as mentioned in Table I) were prepared which were half-neutralized lime. Table I shows the the results with one soil sample (113p.)*

TABLE I

Uptake of base by soil No. 113p from half-neutralized buffer solutions of concentrations 0.06N and 0.04N at different pH values

Organic acid	pH	0.06N	0.04N
Monochloroacetic acid	2.9	-3.7	-3.7
Acetic acid	4.6	-0.66	-0.66
P-nitrophenol	7.1	+1.27	+1.27
Phenol	9.8	+6.90	+6.90

The results in Table I show that within the limits of experimental error the uptakes of base at the two acid concentrations of the buffer solutions are practically the same.

Determination of total exchangeable bases

The total exchangeable bases of the soil were determined by the method of Williams [1929]. The observed figures of the exchangeable bases were corrected for the carbonate contents of the soils wherever the soils contained measurable amounts of carbonate. A correction was made for the exchangeable bases in the reagents employed.

Determination of saturation capacity at pH 7.0

The saturation capacities at pH 7.0 were determined by the barium acetate-ammonium chloride method of Parker [1929].

Determination of organic carbon

The organic carbon was determined by the modified method of Walling [1935].

*The pH values at half-neutralized points of the acids at these concentrations will be the same following the equation $pH = pKa$ at the half-neutralized points of the acids.

Electrodialysis of soils

The soils were electro dialysed in a three-chambered electro dialysis vessel. Substances were kept in the middle chamber and electro dialysis was carried out until the liquid at the cathode was neutral.

RESULTS AND DISCUSSION

Exchangeable bases, saturation capacities and base saturation

Table II shows the m. eq. of exchangeable bases (x), saturation capacities and percentage base saturation ($x/y \times 100$), calculated on oven-dry basis.

TABLE II

Exchangeable bases in m. eq. per cent, saturation capacities in m. eq. per cent and per cent base saturation
(Calculated on oven-dry basis)

Locality	Soil No.	Depth	x	y	$x/y \times 100$	pH
Bhawa, Manbhum, Bihar	81p	0—1 ft. 6 in. .	3.58	6.95	51.51	4.9
	82p	1 ft. 6 in.—2 ft. 3 in.	4.33	8.24	52.55	5.6
	83p	2 ft. 3 in.—3 ft. 6 in.	5.91	8.80	67.16	5.8
	84p	3 ft. 6 in.—4 ft. 11 in.	5.16	8.85	58.31	5.9
	85p	4 ft. 11 in. below	5.29	8.45	62.60	5.8
Bhaktang, Khurda Town, Orissa	106p	0—1 ft. .	7.06	nd	..	4.5
	107p	1 ft.—2 ft. .	6.71	8.67	77.39	4.8
	108p	2 ft.—8 ft. 6 in.	8.26	10.95	75.44	5.3
	109p	8 ft. 6 in.—10 ft.	7.07	7.80	90.64	5.9
	110p	30 ft.—50 ft. .	4.17	4.49	97.33	6.8
Bhaktang, Midnapur, Bengal	112p	0—4 in. .	5.95	11.7	50.85	5.8
	113p	4 in.—3 ft. 4 in.	5.99	9.83	60.93	5.6
	114p	3 ft. 4 in.—4 ft.	4.98	7.19	69.26	5.3
	115p	7 ft.—8 ft. .	4.15	9.83	43.22	4.5
Bhaktang, Khasi Hills, Assam	124p	0—7 in. .	1.33	2.9	55.65	4.0
	125p	7 in.—10 in. .	3.00	4.66	64.38	4.6
	126p	10 in.—4 ft. .	3.86	4.12	93.68	5.0
	127p	10 ft. below .	6.74	nd	...	7.2

The data in Table II show that in the case of Hathwara (Manb Bihar), the percentages base-saturation increase down the profile and remain fairly constant. In the case of Jhinkartangi (Khurda Farm, Orissa) percentages base-saturation remain fairly constant down the profile and increase at greater depths. In the case of Lalgargh (Midnapur, Bengal) percentages base-saturation increase down the profile and then decrease at greater depth. In the case of soils of Cheerapunji (Khasi Hills, Assam) x/y values increase regularly as the depth of the profile increases.

Study of buffer curves

Table III gives the data on the m. eq. of base taken up by 100 g oven-dry soils at pH values 1.3, 2.9, 4.6, 7.1, 9.8 and 12.5.

TABLE III

M. eq. of base taken up by 100 gm. of oven-dry soil at different pH values.

Locality	Soil No.	Depth	pH 1.3	pH 2.9	pH 4.6	pH 7.1	pH 9.8
Hathwara, Manbhum, Bihar	81p	0—1 ft. 6in. .	—9.1	—4.1	—0.59	1.4	9.8
	82p	1 ft. 6in.—2ft. 3 in.	—8.2	—4.8	—1.2	0.91	11.5
	83p	2 ft. 3in.—3ft. 6 in.	—8.7	—5.0	—1.4	0.90	11.8
	84p	3 ft. 6in.—4ft. 11 in.	—7.4	—4.8	—1.0	0.90	10.6
	85p	4 ft. 11 in. below	—7.6	—2.9	—0.71	0.69	5.1
Jhinkartangi, Khurda Town, Puri, Orissa	106p	0—1 ft. .	—25.8	—5.2	—0.07	7.7	25.6
	107p	1 ft.—2 ft. .	—10.7	—7.0	—0.56	4.9	22.3
	108p	2 ft.—8 ft. 6 in.	—10.7	—6.9	—1.4	3.6	23.5
	109p	8 ft. 6 in.—10 ft.	—11.4	—7.6	—1.7	2.5	21.3
	110p	From the diggings of a well	—11.1	—2.8	—1.4	0.13	4.9
Lalgargh, Midnapur, Bengal	112p	0—4 in. .	—11.0	—5.8	—1.1	1.8	12.0
	113p	4 in.—3ft. 4in.	—8.0	—3.7	—0.66	1.3	6.9
	114p	3 ft. 4in.—4ft.	—7.0	—1.9	—0.18	0.89	4.1
	115p	7 ft.—8 ft. .	—6.9	—1.6	—0.11	1.9	7.5
Cheerapunji, Khasi Hills, Assam	124p	0—7 in. .	—2.2	—0.82	0.18	1.8	6.9
	125p	7 in.—10 in..	—6.4	—3.6	—0.47	3.3	11.1
	126p	10 in.—4 ft..	—6.9	—3.8	—1.4	1.7	7.6
	127p	10 ft. below .	—8.9	—5.7	—4.2	1.0	0.64

TABLE III.—*contd.*

Locality	Soil No.	Depth	pH 1·3	pH 2·9	pH 4·6	pH 7·1	pH 9·8	pH 12·5
Jalpaiguri, Khasi and Jaintia Hills, Assam	134p	0—6 in.	—9·3	—5·5	—0·26	9·4	32·3	39·7
	135p	6 in.—3ft. 6in.	—8·3	—4·1	0·48	14·1	33·8	44·0
	136p	3 ft. 6 in.—4 ft. 2 in.	—5·5	—2·3	2·0	15·5	39·9	58·2
	137p	4 ft. 2 in.—6 ft.	—6·6	—4·3	0·19	8·9	31·9	44·7
Jalpaiguri, Gau- hati, Assam	139p	0—6 in.	—10·8	—6·3	—1·1	3·4	17·4	26·9
	140p	6 in.—11 ft.	—7·2	—4·1	—0·18	4·7	15·2	25·6
	141p	11 ft.—16 ft.	—5·8	—2·3	0·83	4·6	13·2	30·5
	142p	16 ft. below	—4·3	—1·8	—0·18	1·1	5·7	8·0
	143p	From a cut- ting on the top of a hillock	—7·0	—2·4	0·84	5·0	16·2	31·9
Jalpaiguri, Tura, Hills, Assam	152p	0—10 in.	—10·8	—6·6	—0·37	9·5	33·0	39·5
	153p	10 in.—2 ft.	—9·2	—5·4	3·6	18·2	44·2	52·8
	154p	2 ft.—4 ft.	—12·1	—8·4	2·5	15·7	52·2	61·7
Jalpaiguri, Bogra, Bengal	156p	0—1 ft.	—11·8	—4·8	—0·37	2·4	13·7	22·2
	157p	1 ft.—2 ft.	—8·7	—4·9	—0·37	2·3	15·2	27·8
	158p	2 ft.—4 ft.	—11·5	—5·6	—1·1	2·7	17·1	22·5
	159p	12 ft.—25 ft.	—13·8	—5·4	—0·73	1·1	8·8	14·6
	160p	25 ft.—30 ft.	—14·9	—4·5	—0·91	1·1	7·4	13·3
Jalpaiguri, Road, Barind Tract, Ajshahi, Bengal	162p	0—1 ft. 10 in.	—9·1	—5·0	—0·37	1·5	10·0	17·4
	163p	1 ft. 10 in.— 2 ft. 3 in.	—12·6	—6·2	—0·75	1·7	15·0	25·3
	164p	2 ft. 3 in.—4 ft.	—15·4	—7·8	—1·7	0·74	13·4	26·2

Table III shows that for soil samples 81p-85p (Hathwara Farm, Manbhum, Bihar) between pH ranges 1·3 to 7·1, the buffer capacities of all the soil samples are almost equal, but beyond pH 7·1, 85p (4 ft. 11 in. below) has least buffer action, whilst with soils 81p-83p, the buffer action increases as the depth of the soil sample in the profile layer increases. Soil No. 84p (3 ft. 6 in.—4 ft. 11 in.) is intermediate in buffer capacity.

For soil samples 106p-110p (Jhinkartangi, Khurda Farm, Puri, Or.) the buffer capacities in general decrease as we pass from the top layer downwards. At higher pH regions 108p possesses greater buffer action than 106p. With samples 112p-115p (Lalgarh, Midnapur, Bengal), the buffer capacities decrease as the depths of the profile increase. With soil samples 117p-127p (Cheerapunji, Khasi Hills, Assam), the top soil 124p has moderate buffer capacity whilst soils of intermediate layers 125p (7-10 in.) and 126p (10-14 ft.) possess greater buffer action than top soil. Soil of the lowest layer of profile 127p (10 in. below) possesses least buffer action. With samples 134p-137p (Nongpoh, Khasi and Jaintia Hills, Assam), the top soil 134p is found to possess the least buffer action, whilst the soil from third layer 136p (3 ft. 6 in.—4 ft. 2 in.), has got maximum buffer action. Soil No. 135p (4 ft. 2 in.—6 ft.) has got almost the same buffer action as that of 135p. The buffer capacities of soil samples 139p-141p (Uzanbazar, Gauhati, Assam) are almost equal, 142p of the same profile being an exception. With samples 152p-154p (Babupura, Tura, Garo Hills, Assam), 152p (10 ft.) has got least buffer action, whilst 154p (2 ft.—4 ft.) has got the maximum buffer action. With soil samples 156p-160p (Sultanganj, Bogra, Bengal), it is found that soil samples from intermediate layers 157p (1 ft.-2 ft.) and 158p (2 ft.—4 ft.) possess greater buffer action, whilst soil samples from bottom layers 159p (12 ft.—25 ft.) and 160p (25 ft.—30 ft.) possess less buffer action. With soil samples 162p-164p (Khetur Road, Barind Tract, Rajshahi, Bengal), the top soil samples 162p (0-1 ft. 10 in.) has got the least buffer action, whilst soil samples from 2nd and 3rd layers of the profile, 163p (1 ft. 10 in.—3 in.) and 164p (2 ft. 3 in.—4 ft.), possess almost equal buffer capacities.

The buffer action is due to buffer materials present in the soil and the mode of variation of buffer capacity shows the manner of accumulation of buffering materials at different layers of the profiles. Within certain limits of approximations, the soil profiles studied can be classified from the mode of variations of buffer capacities at different layers of the profiles into the following four classes :

- (1) Cases where the buffer action in general increases down the profile, e.g. profiles of Tura (Garo Hills, Assam) and Rajshahi (Bengal).
- (2) Cases where the buffer action in general decreases down the profile, e.g. profiles of Khurda Road (Orissa), and of Midnapur (Bengal).
- (3) Cases where the buffer action in general remains constant down the profile, e.g. profile of Gauhati (Assam).
- (4) Cases where the buffer action shows a maximum value at an intermediate depth of the profile, e.g. the profiles of Cheerapunji and Nongpoh (Assam) and of Bogra (Bengal).

In agreement with the observations made by Raychaudhuri and Nandymazumdar [1939], it is found that almost all the buffer curves* indicate more or less definite inflexions at pH 9.8 and frequently a second inflexion either at pH 2.9 or at pH 4.6.

The variations of the buffer values (dB/dpH) of the soil samples at these pH values [Van Slyke, 1922] do not show any regularity.

*Not shown in figures

Comparison of pH values of soils obtained by different methods

Table IV summarizes the data on the pH values of soils obtained by Kuhn's method, by Quinhydrone electrode and from the intersection of buffer curves with the line of zero uptake of base.

TABLE IV

Comparison of pH values of soils obtained by different methods

Locality		Soil No.	Depth	pH by		
				Kuhn's method	Quinhydrone electrode	Buffer curve
Bhawanra, Bihar	Manbhum,	81p	0—1 ft. 6 in. .	4.9	5.55	5.65
		82p	1 ft. 6 in.—2 ft. 3 in.	5.6	5.71	5.75
		83p	2 ft. 3 in.—3 ft. 6 in.	5.8	5.65	5.75
		84p	3 ft. 6 in.—4 ft. 11 in.	5.9	5.81	6.35
		85p	4 ft. 11 in. below	5.8	6.16	5.6
Tunkartangi, Town, Puri, Orissa	Khurda	106p	0—1 ft. .	4.5	nd	4.55
		107p	1 ft.—2 ft. .	4.8	5.92	4.75
		108p	2 ft.—8 ft. 6 in.	5.3	5.95	5.25
		109p	8 ft. 6 in.—10 ft.	5.9	6.18	5.55
		110p	From the digging of a well	6.8	6.58	6.70
Ulgharh, Bengal	Midnapur,	112p	0—4 in. .	5.8	6.41	5.15
		113p	4 in.—3 ft. 4 in.	5.6	5.81	5.6
		114p	3 ft. 4 in.—4 ft.	5.3	5.96	5.7
		115p	7 ft.—8 ft. .	4.5	5.01	5.25
Jorapunji, Assam	Khasi Hills,	124p	0—7 in. .	4.8	5.24	3.75
		125p	7 in.—10 in. .	4.7	5.21	4.9
		126p	10 in.—4 ft. .	5.0	5.18	6.05
		127p	10 ft. below .	7.2	nd	8.2

TABLE IV—*contd.*

Locality	Soil No.	Depth	pH by		
			Kuhn's method	Quinhydrone electrode	Buffer curve
Nongpoh, Khasi and Jaintia Hills, Assam	134p	0—6 in.	4.9	4.84	4.8
	135p	6 in.—3 ft. 6 in.	4.7	5.06	4.8
	136p	3 ft. 6 in.—4 ft. 2 in.	4.4	4.59	3.8
	137p	4 ft. 2 in.—6 ft.	4.5	4.55	4.8
Uzanbazar, Gauhati, Assam	139p	0—6 in.	5.2	5.79	5.8
	140p	6 in.—11 ft.	4.8	5.29	4.8
	141p	11 ft.—16 ft.	4.8	5.22	4.8
	142p	16 ft. below	5.0	5.19	5.8
	143p	From a cutting on the top of a hillock	4.7	4.83	4.8
Babupara, Tura, Garo Hills, Assam	152p	0—10 in.	4.8	5.09	4.8
	153p	10 in.—2 ft.	4.6	4.91	4.8
	154p	2 ft.—4 ft.	4.7	5.29	4.8
Sultanganj, Bogra, Bengal	156p	0—1 ft.	5.6	6.55	5.8
	157p	1 ft.—2 ft.	5.5	6.72	5.8
	158p	2 ft.—4 ft.	5.5	6.32	5.8
	159p	12 ft.—25 ft.	5.7	6.58	6.8
	160p	25 ft.—30 ft.	5.8	6.52	6.8
Khetur Road, Barind Tract, Rajshahi, Bengal	162p	0—1 ft. 10 in.	5.6	6.78	6.8
	163p	1 ft. 10 in.—2 ft. 3 in.	5.6	6.22	6.8
	164p	2 ft. 3 in.—4 ft.	6.2	6.52	6.8

From Mattson's [1937] point of view the pH at which the buffer curve intersect the line of zero-adsorption should correspond to the 'equi-

at'. The results in general show that the pH values obtained by the an's method and in general those indicated by the point of intersection of buffer curves with the line of zero uptake of base are both slightly lower than those obtained by the quinhydrone electrode method. In general, the point of intersection of buffer curves with the line of zero uptake of base corresponds more closely with the pH values obtained by the Kuhn's method than with the quinhydrone pH .

buffer curves of minerals

During the course of the present work it was felt desirable to compare pH values for the uptake of base by the profile samples at different pH values with the corresponding uptake by some naturally occurring minerals like limonite, bauxite, halloysite, kaolin and montmorillonite (Table V).

TABLE V

Uptake of base in m. eq. per 100 gm. of minerals at different pH values

Minerals	pH 1.3	pH 2.9	pH 4.6	pH 7.1	pH 9.8	pH 12.5
bauxite .	-0.94	-0.51	-0.26	+2.2	+8.3	+18.0
halloysite .	-8.5	-3.6	-2.6	+0.38	+9.5	+34.3
kaolin .	0.00	+4.2	+4.2	+4.5	+7.0	+18.0
limonite .	-1.5	-1.8	-3.1	-0.88	+7.5	+11.8
montmorillonite	-5.1	-1.7	-1.5	+0.38	+4.5	+10.5

Fig. 1 shows the nature of the buffer curves obtained. The curves show that the mineral halloysite possesses higher buffer capacities than kaolin and the curves are typically S-shaped ones, and show inflexions at pH 2.9. The mineral limonite shows an interesting behaviour in that, contrary to expectations, the uptake of base at pH 4.6 by this substance is higher than the uptake of base at pH 2.9. This is probably due to complex sparingly soluble compounds being formed whose solubility varies differently with variation in pH values.

buffer curves of Merck's humic acid

The results are shown in Table VI.

TABLE VI

Uptake of base in m. eq. per 100 gm. of humic acid at different pH values

	pH 1.3	pH 2.9	pH 4.6	pH 7.1	pH 9.8	pH 12.5
humic acid .	-39.7	-27.9	+53.7	+123.6	+268.3	+406.9

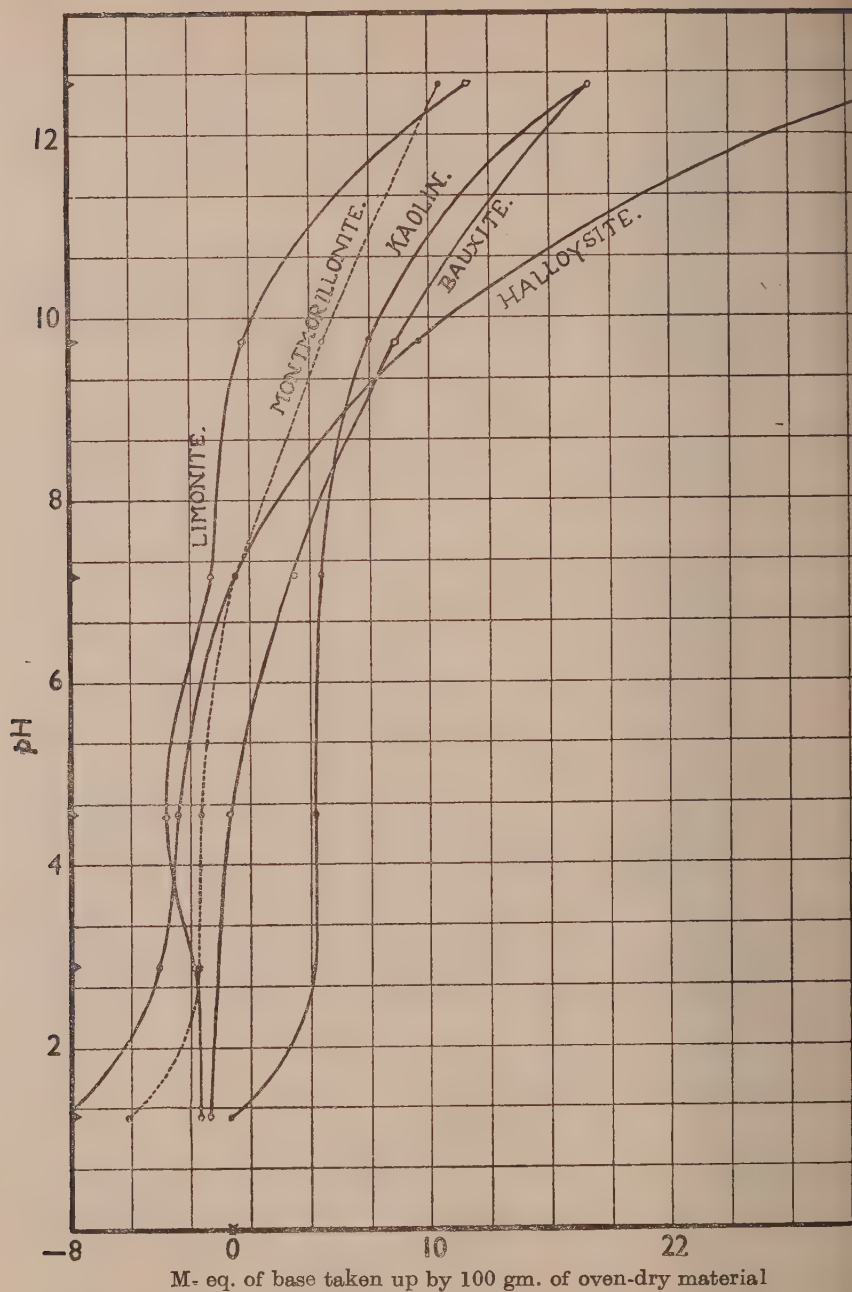


FIG. 1

The results are plotted graphically in Fig. 2. The curve shows inflexion at pH 4.6. Raychaudhuri and Nandymazumdar [1939] previously suggested that the inflexion of buffer curves in this region might be due to

nce of free alumina, since at about pH 4.6, alumina tends to go into sol state. The present observation, however, suggests that this in-

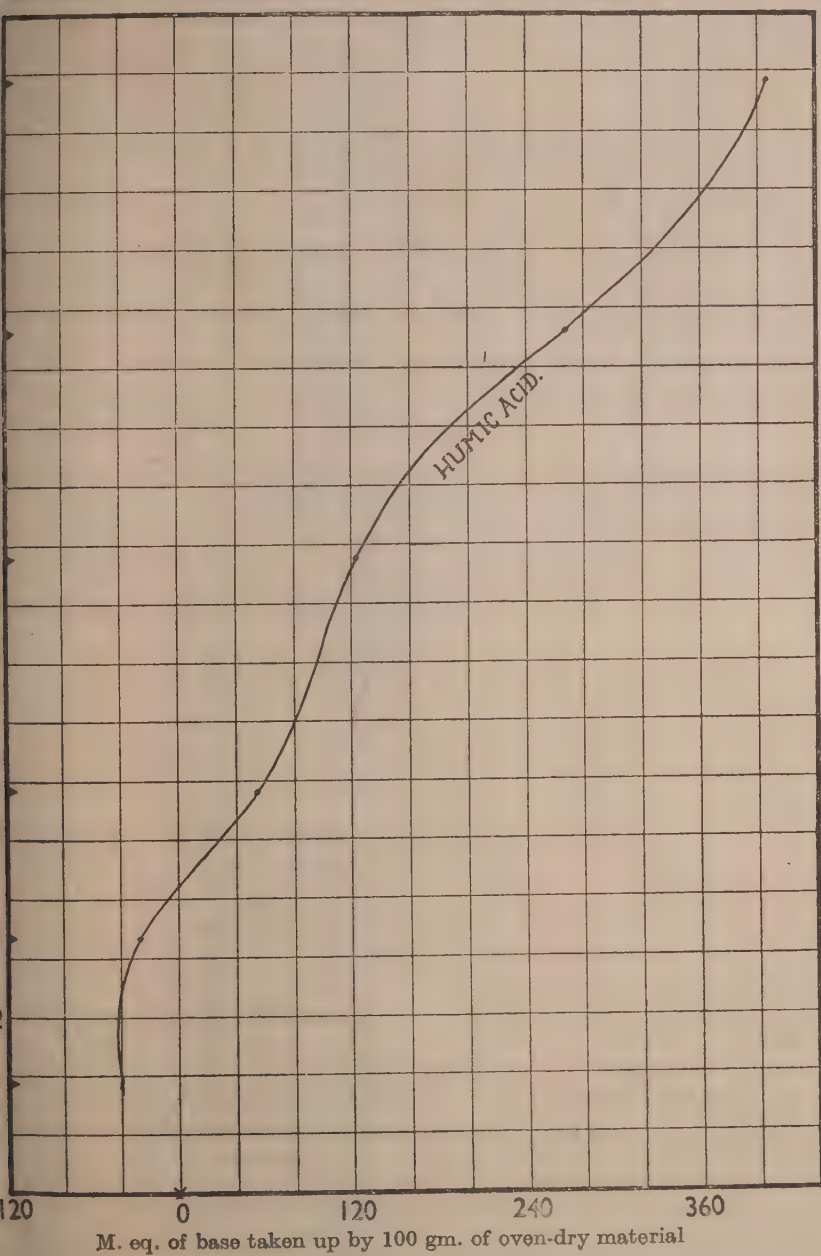


FIG. 2

flexion at about pH 4.6 might be due, at least to some extent, to organic matter which is present in the soil. This point requires further detailed investigations.

Comparison of buffer curves by using different alkalies as the adsorbed base

In the course of the investigation on the inflexion points of the buffer curves, drawn with calcium buffers at the three pH values—2.9, 4.6, and 7.1—it was thought that the solubilities of the resulting calcium silicates might have something to do with the inflexions of the curves.

The adsorption of base, according to Wiegner [1931], depends on the hydration of the cation, whilst according to the point of view of Mukherjee [1922], the adsorbability of cation would be determined by its valency and electrical mobility. According to these hypotheses we would expect the buffer capacity of the soil to follow the order of lyotropic series, that is $K > Na > Li$.

Table VII gives the data on the relative adsorbabilities of the cations Ca and Na for three air-dry soils (85p, 113p and 124p), at different pH values.

TABLE VII

Uptake of calcium and sodium [as $Ca(OH)_2$ and $NaOH$] by three soils at different pH values

pH		85p	113p	124p
1.3	{ Ca	-7.6	-8.0	-2.0
	{ Na	-7.0	-7.2	-2.0
2.9	{ Ca	-2.9	-3.7	-8.0
	{ Na	-3.1	-4.7	-1.0
4.6	{ Ca	-0.71	-0.66	0.0
	{ Na	-0.22	-0.74	-0.0
7.1	{ Ca	0.69	1.3	1.0
	{ Na	0.22	0.68	1.0
9.8	{ Ca	5.11	6.9	6.0
	{ Na	3.88	5.0	4.0

In general the data in Table VII uphold Mukherjee's [1922] theory of ionic adsorption, in that the adsorption of Ca is greater than that of sodium in most cases, with a few exceptions are, however, noticeable. For instance, in the case of pH 1.3, the negative adsorption of Na is greater than that of calcium, which means that positive adsorption of sodium is higher. Another exception is shown at pH 4.6 in the case of soil No. 85p, where the relative adsorption of two bases is nearly equal.

Comparison of bases taken up by soils at different pH values

Table VIII gives the data on the uptake of m. eq. of different bases by 100 gm. of air-dry soil (No. 113p), the results being expressed on oven-dry basis as usual.

TABLE VIII

M. eq. base taken up by 100 gm. of oven-dry soil No. 113p

pH	Li	Na	K	Ca
1.3	—8.3	—7.2	—7.0	—8.0
2.9	—4.5	—3.7	—3.2	—3.1
4.6	—1.6	—0.74	—0.72	—0.66
7.1	0.65	0.68	0.81	1.27
9.8	5.7	5.0	6.2	6.9
12.5	17.6	17.6	17.6	17.6
(Baryta)				

Table IX, on the other hand, gives the data on the uptake of base by 100 gm. of air-dry electrodialysed soil (No. 113p), results being expressed on oven-dry basis. In the case of electrodialysed soil it was found necessary to centrifuge the mixture in order to get a clear supernatant liquid. The percentage of moisture in the electrodialysed soil was 2.93.

TABLE IX

M. eq. of base taken up by 100 gm. of electrodialysed soil No. 113p

pH	Li	Na	K	Ca
1.3	—3.5	—3.4	—3.3	—3.2
2.9	—1.32	—1.34	0.4	1.3
4.6	—0.38	2.6	3.3	0.4
7.1	8.4	8.5	8.7	9.0
9.8	12.9	14.5	15.0	16.1
12.5	28.8	28.8	28.8	28.8
(Baryta)				

The data in Tables VIII and IX show that the adsorption of metal cations is generally in the order $\text{Ca} > \text{K} > \text{Na} > \text{Li}$. Ca is divalent and hence it could be adsorbed to the maximum extent by the negatively charged clay. The adsorption of K, Na and Li follows the order of solvation of these ions and hence is in agreement with the lyotropic series. There is, however, one peculiarity to be noticed in the relative order of curves. It will be found that the relative difference in the adsorption of different cations are maximum at pH 4.6, specially for the electrodialysed soil. The relative difference between the adsorbabilities by the different cations are fairly considerable at pH 9.8, whilst between pH ranges 4.6 to 1.3, the difference between the adsorbabilities narrows down to zero. Also at pH 7.1, the adsorbabilities of different cations are practically the same.

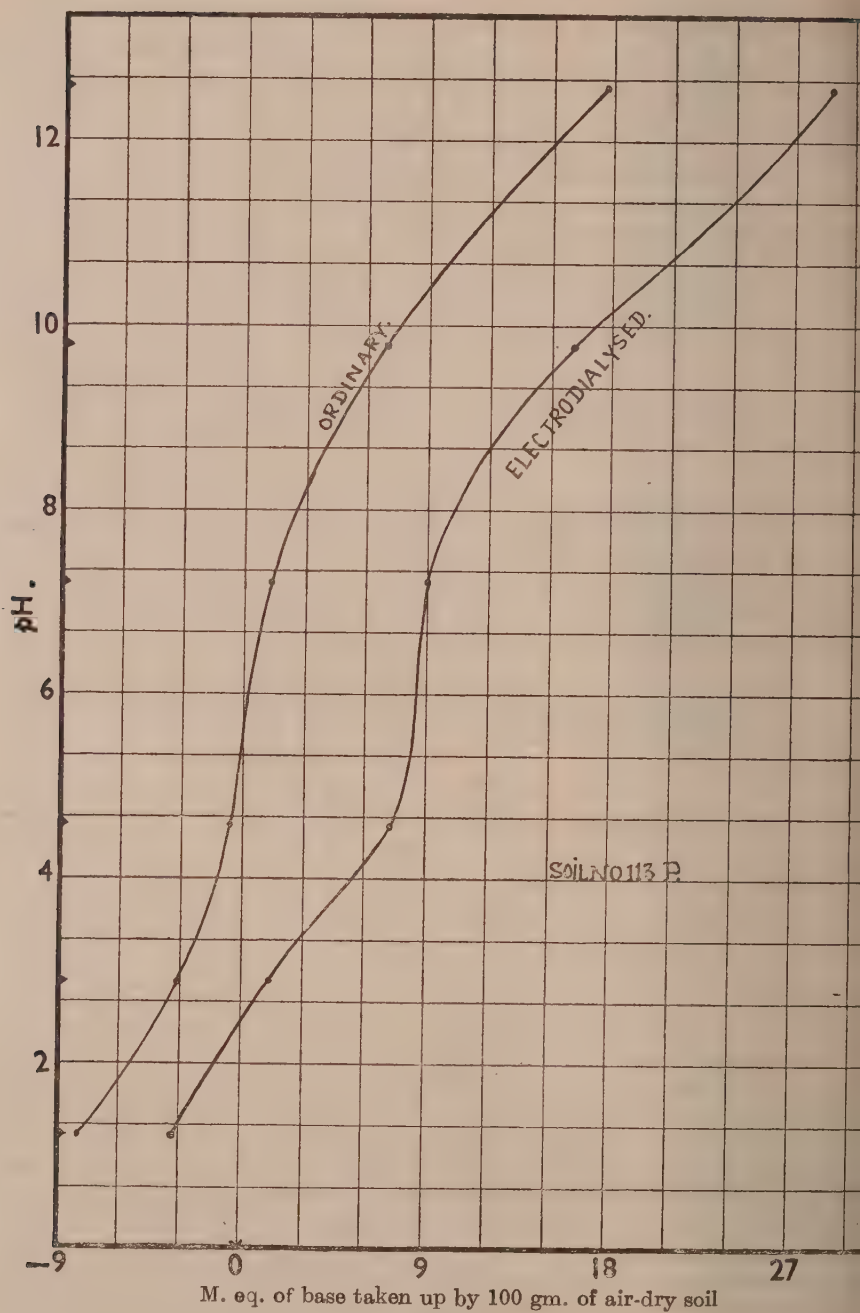


FIG. 3

Uptake of calcium under different conditions

Table X shows the results on the uptake of lime by two soil samples 113p and 152p before and after electro dialysis. The results with soil 113p before and after electro dialysis are plotted graphically in Fig. 3. The buffer curves of 113p before and after electro dialysis show the same general behaviour.

TABLE X

Uptake of lime by soil samples 113p and 152p before and after electro dialysis

pH	113p		152p	
	Ordinary air-dry soil	Electrodialysed soil	Ordinary air-dry soil	Electrodialysed soil
1.3	—8.0	—3.2	—10.8	—3.20
2.9	—3.1	1.3	—6.6	0.53
4.6	—0.66	7.4	—0.37	5.6
7.1	1.3	9.0	9.5	18.3
9.8	6.9	16.1	33.0	37.3
12.5	17.6	28.8	39.5	52.8

The percentage of moisture of the electro dialysed soil 113p was 2.93 that of the electro dialysed soil 152p was 3.83. As is to be expected, the buffer curves of the ordinary air-dry soil and of the same soil after it has been electro dialysed run almost parallel to each other, the reason being that electro dialysis of soils causes replacement of ordinary exchangeable bases by hydrogen.

SUMMARY AND CONCLUSIONS

In the present paper the chief base-exchange properties of a number of profile samples of red and lateritic soils of India have been studied. This includes the study of pH, percentages of air-dry moisture, total exchangeable bases, degree of saturation and buffer curves. Buffer curves of minerals like kaolinite, limonite, halloysite, kaolin, montmorillonite and of Merck's humic acid have been studied. The relative adsorbabilities of cations calcium and magnesium by the soils and the milli-equivalents of bases Li, Na, K and Ca taken up at different pH values have also been determined. Lastly, the determination of percentages of organic carbon of a number of soils and its influence on the nature of buffer curves have been studied. The main general conclusions are :—

1. Percentages of base-saturation of the profile samples generally increase with depth in the profile. In some cases the percentage base-saturation attains a maximum at an intermediate depth.

2. Within certain degree of approximation the soil profiles studied are classified from the mode of variation of buffer capacities at different layers into four classes.

3. Almost all the buffer curves indicate more or less definite inflexion at pH 9.8 and frequently a second inflexion either at pH 2.9 or at pH 4.6.

4. A comparison was made of the pH values obtained by the Kuhn's method, the quinhydrone electrode and that obtained from the intersection of the buffer curves with the line of zero uptake of bases. In general the points of intersection of the buffer curves with the line of zero uptake of bases corresponds very closely with the pH values obtained by Kuhn's method. Both these pH values are slightly lower than those obtained by quinhydrone electrode method.

5. The nature of the buffer curves obtained with limonite, bauxite, halloysite, kaolin and montmorillonite shows that the mineral halloysite possesses higher buffer capacities than kaolin. All the curves are typical S-shaped ones and show inflexions at pH 2.9. The mineral limonite shows an interesting behaviour, in that contrary to expectations the uptake of base at pH 4.6 by this substance is higher than the uptake of base at pH 2.9.

6. Humic acid shows a very high buffer capacity, and the buffer curve of the humic acid shows an inflexion at pH 4.6.

7. The relative adsorbabilities of the cations calcium and sodium on three air-dry soils (85p, 113p and 124p) at different pH values have been compared. The adsorption of calcium is greater than that of sodium.

8. The buffer curves of ordinary air-dry soil 113p and of the same soil after electrodialysis with the four bases Ca, Li, Na and K are nearly coincident, and the lyotropic series holds good in practically all the cases.

9. The buffer curves of ordinary air-dry soil and the same soil after electrodialysis has been electrodialysed run almost parallel to each other.

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STUDIES ON THE CHEMICAL CONSTITUENTS OF INDIAN LATERITIC AND RED SOILS

DETERMINATION OF THE PERCENTAGE OF CLAY, MAXIMUM
WATER-HOLDING CAPACITY AND OF FREE IRON OXIDE, FREE
ALUMINA AND FREE SILICA OF LOWERMOST LAYERS OF
PROFILE SAMPLES

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(With two text-figures)

RAYCHAUDHURI and Sulaiman [1940] have laid stress on the importance of determining the percentages of free silica and of free alumina and free iron oxide in the case of lateritic soils. These authors have determined percentages of free sesquioxides in Indian lateritic and red soils following methods devised by Hardy [1931] and by Drosdoff and Truog [1935]. Percentages of free iron oxides obtained by Hardy's method were found to be much smaller than those obtained by the method of Drosdoff and Truog. But the percentages of free alumina obtained by Hardy's procedure are much larger than those obtained by the other method. More recently Hardy [1939] and Truog [1936] have modified their previous methods. The unpublished work of Sulaiman shows that the percentages of free alumina obtained by the modified methods of Hardy and of Truog are very nearly equal. In these two methods, however, the method devised by Truog and co-workers got special advantage in that it is possible, by this method, to obtain soil residues free from iron and aluminium oxides. Raychaudhuri, Sulaiman and Suraychaudhuri [1941], in a recently published work, have shown that the presence of free sesquioxides and free silica in Indian red soils has considerable influence on the buffer capacities of the soils in that the buffer curves of the residues after removal of the free silica and free sesquioxide components become steeper as compared to those of the corresponding original soils.

In connection with the work on lateritic and red soils of India, it was desirable to examine the physico-chemical properties of soil samples of the lowermost layers of profiles of these soil types and find out correlation, if any, between the physico-chemical properties of soils from bottom layers of these profiles with the nature and quantities of the mineral assemblages present in the soils and the parent material, the fundamental assumption being that the lowermost layer could be least affected by weathering agencies [Bonnett, 1939].

EXPERIMENTAL

The percentages of clay in the soil samples were determined by the method of Robinson [1933]. The maximum water-holding capacities of the samples were determined by following essentially the procedure devised by Kee and Raczkowski [1921]. The percentages of amorphous products of weathering, e.g. free Fe_2O_3 , free Al_2O_3 and free SiO_2 , were determined by the method of Drosdoff and Truog [1935], subsequently modified by Truog and coworkers [1937]. The percentages of ferro-magnesian minerals present in the soils were determined by following the procedure suggested by Hendrick and Newell [1923]. The results are shown in Table I where the percentages of the clay fraction in the soil and the percentages by volume of ferro-magnesian minerals (determined by the procedure used by Jeffries [1937]) are also included.

TABLE I

Percentages of clay fractions, of maximum water-holding capacities, of free iron oxide, of free Al_2O_3 and of free SiO_2 of lowermost layer of the profile samples

Locality	Depth	Soil No.	Per cent clay fraction	Per cent max. water-holding capacity	Per cent free Fe_2O_3	Per cent free Al_2O_3	Per cent free SiO_2	Per cent ferro-magnesian minerals
Hathwara, Manbhum, Bihar	4 ft. 11 in. below	85 p	24.2	58.2	2.86	0.53	0.550	
Putida, Singbhum, Bihar	2 ft. 9 in.—4 ft.	89 p	20.6	61.9	6.49	1.01	1.095	
Baralota, Daltonganj, Bihar	4 ft.—5 ft.	97 p	12.4	57.0	1.07	0.31	0.924	
Tangl, Cuttack, Orissa	2 ft.—4 ft.	100 p	18.1	49.9	11.68	2.38	1.128	
Dhanmandal, Cuttack, Orissa	5 in.—4 ft.	102 p	67.10	72.0	9.86	2.22	0.624	
Kapileswar, Bhubaneswar, Orissa	2 ft. 11 in.—4 ft.	104 p	28.3	50.0	10.91	1.48	0.835	
Jhinkar, Tangl, Khurda Town, Orissa	8 ft. 6 in.—10 ft.	109 p	23.0	n. d.	8.38	1.89	0.780	
Lalgarh, Midnapore, Bengal	3 ft. 4 in.—4 ft.	114 p	27.6	54.9	3.93	1.34	0.645	
Midnapore (Bed of Cossye river)	Bed of Cossye river	117 p	48.4	67.0	1.06	0.42	0.775	
Malda, Midnapore	40 ft. below	118 p	27.6	66.1	4.50	0.61	0.616	
Mawphlang, Khasi Hills, Assam	2 ft. 11 in.—4 ft.	122 p	37.0	71.3	10.32	1.57	0.577	
Upper Chandmari, Tura, Garo Hills, Assam	2 ft. 8 in.—4 ft.	147 p	8.20	50.2	1.46	0.49	0.779	

Fig. 1 shows graphically the correlation between the percentages of clay fractions of the soil samples and their maximum water-holding capacities. It is found that there is fair linear relationship between the two. A particular

interesting case is that with sample 122 p (Mawphlang, Khasi Hills, Assam), where the maximum water-holding capacity is as high as 71.3, whereas the percentage of clay fraction is only 37.0. Similarly also, the sample 118 p shows a comparatively high value for maximum water-holding capacity (66.1), whilst the percentage of clay fraction is as low as 27.6.

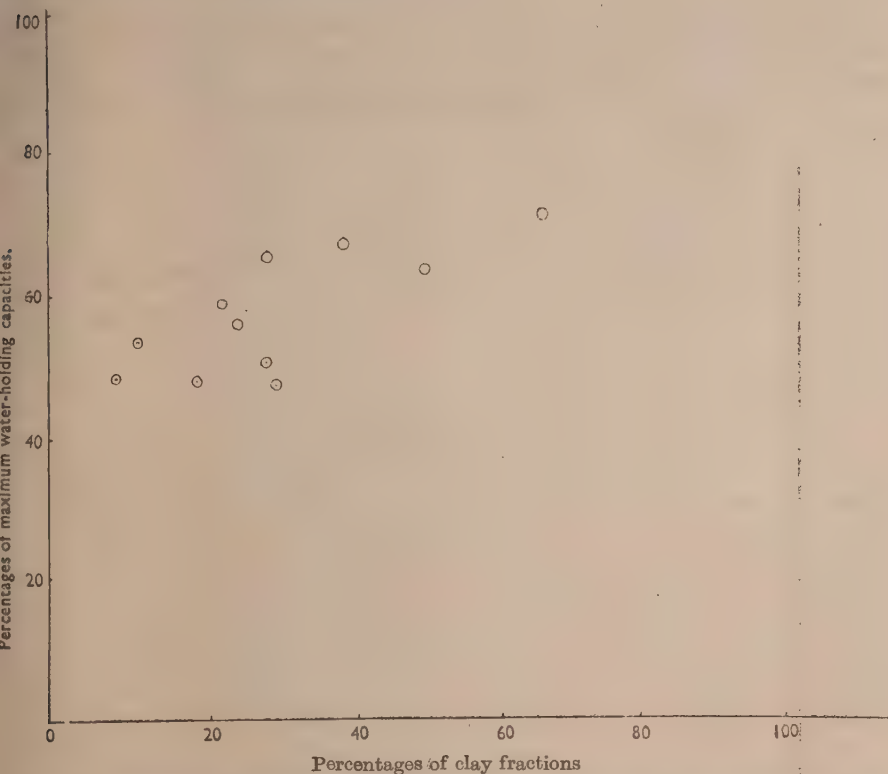


FIG. 1. Correlation between the percentages of clay fractions and their maximum water-holding capacities

Fig. 2 was drawn to show the relationship between the percentages of free alumina and of free iron oxide, with the percentages of ferro-magnesian minerals. It is generally to be expected that the greater the proportion of ferro-magnesian minerals, the less would be the proportions of free alumina and of free iron oxide. The figure shows that this is generally the case. Table I shows that the amount of free silica in the soil samples is very low in all cases.

SUMMARY

1. A comparative study has been made of the physico-chemical and mineralogical properties of the bottom layer samples of some red and lateritic

soil profiles. The determinations made in this connection are maximum water holding capacities and percentages of clay, of free Fe_2O_3 , free Al_2O_3 , SiO_2 , and of ferro-magnesian minerals present in the soils.

2. A fair linear relationship was observed between the percentages of clay fraction of soil samples from the bottom layers of the profiles and the maximum water-holding capacities.

3. In general it has been found that the greater the proportion of ferro-magnesian minerals, the less are the percentages of free sesquioxides in samples from the bottom layers.

4. The amount of free silica in the samples of the bottom layers is very low in all cases.

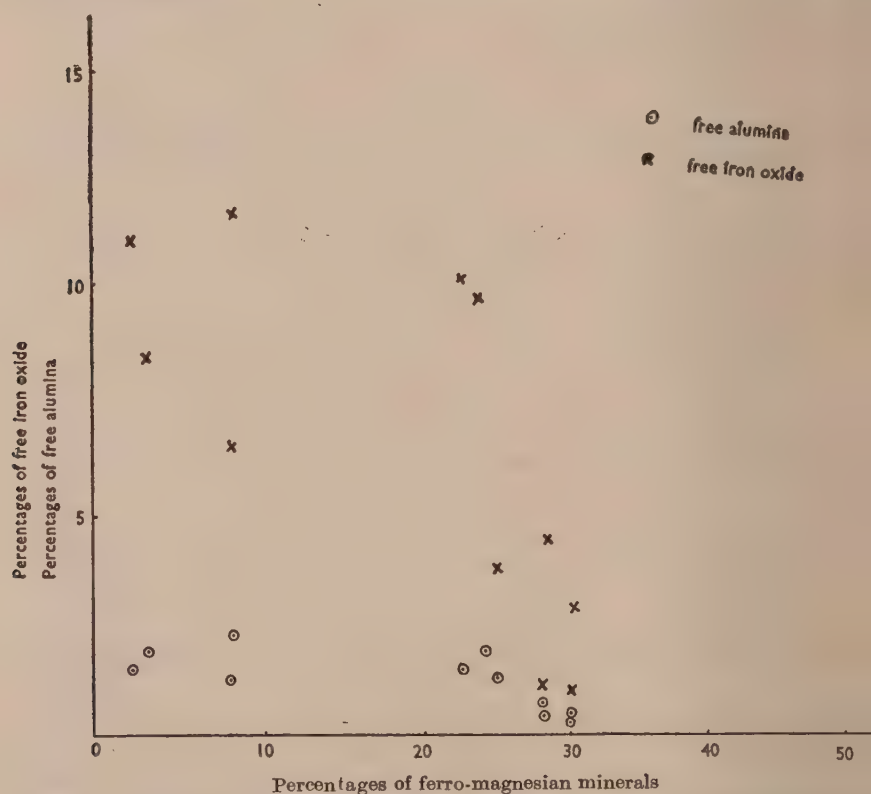


FIG. 2. Relationship between the percentages of free iron oxide and free alumina with ferro-magnesian minerals

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UTILIZATION OF PRESS-MUD, CANE-TRASH AND BAGASSE IN THE CANE FIELDS

I. COMPOSTING BY AEROBIC DECOMPOSITION

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CONTINUOUS cropping of virgin land without periodical addition of any manure is certain to result in a rapid and continuous fall in the total organic matter, nitrogen and moisture-retaining capacity of the soil. Consequently, there will be a reduction in quality as well as quantity of successive crops. In order to safeguard against this, the ingredients taken out of the soil by any crop should be replaced so that the next crop may not suffer due to their absence. The proportion of the essential constituents of the soil can be maintained by the addition of synthetic fertilizers when the deficiency is in nitrogen, calcium, potash or phosphates, and of farmyard manure, green manure or oil cake if the soil is poor in organic matter also.

It is well known that humus or soil organic matter is responsible for soil fertility in a number of ways such as improved tilth, water-retaining power etc. Its deficiency which cannot be rectified by the addition of artificial fertilizers can be made up only by the supply of organic matter in a readily assimilable form. The vegetable waste product added to the soil must contain sufficient combined nitrogen for rapid decomposition which takes place only in proportion to the available combined nitrogen present.

The deficiency in humus which most Indian soils suffer from, is generally made up by the addition of oil-cake, green manure etc. Since oil-cakes are expensive and not available in sufficient quantity in all parts of India, it is desirable to use cheaper humus-forming materials like straw and other cellulosic material. 'Sheet composting' has been suggested for utilizing these, but it would certainly be better if these materials are composted outside the field before being applied to the land, so that the manure will have preformed humus for immediate utilization by the crop. The ripe compost must be in the form of a finely divided powder, with a C : N ratio as close to 10 : 1 as possible.

As a consequence of the development of sugar industry in India, cane trash, filter press cake and molasses and in some factories bagasse also have become available in large quantities. In many cases, even their disposal—not to speak of their utilization—has become a problem to the sugar factory owner. Considerable work is already being done on utilizing molasses to the best advantage; we have therefore confined our attention to evolving the best method of using press cake, cane trash and bagasse on the land. The following table gives the quantities of cane crushed during the last five seasons in the United Provinces, Bihar and in the whole of India by sulphitation factories.

	United Provinces (in million tons)	Bihar (in million tons)	India (in million tons)
36	5.2	2.14	8.8
37	5.9	2.7	10.2
38	5.4	1.9	8.8
39	3.26	1.36	6.2
40	7.0	3.5	13.1

Season 1938-39 was an unusually poor one and need not be taken into consideration. The average quantities of cane crushed per season will therefore be :

United Provinces	Bihar (in million tons)	India
5.9	2.56	10.2

While 20 per cent of cane forms trash, the quantity collected in sugar factories is usually only 1-1.5 per cent. The amount of filter press cake produced is found from the data available, to be about 2.5 per cent on the weight of cane crushed. The cake fresh from the presses contains about 60 per cent moisture and, therefore, the dry matter in the press cake produced may be taken to be 1 per cent on the weight of cane crushed. On this basis, the disposal of the two products the disposal of which is a problem in Indian sugar factories may be estimated (in tons) per season to be—

	United Provinces	Bihar	India
Trash	59,000	25,600	1,02,000
Filter press cake	1,47,500	64,000	2,55,000
Dry matter in the cake	59,000	25,600	1,02,000

These are enormous quantities of potentially useful materials and it is desirable that attempts should be made to use them in the shape of manure or as fuel from which they are produced. It would be ideal if they could be used directly for this purpose. Sporadic attempts have been made in other countries to use cane trash directly on the fields and allow it to decompose, but without much success. Filter press cake is being used as manure in a number of countries and in India also. In the United States of America, it is reported that press cake is almost exclusively used for manurial purposes, especially on the stubble canes on account of its phosphatic constituents. In most of the factories in the United States of America, it is stated, the cake which comes out of the press is directly transported to the fields and applied to the stubble canes; in others, it is stored on the factory premises in a pit and transported later when dry.

In Mauritius, it is recorded that all the filter press cake produced the average composition of which is given in Table I, is used on fields as manure. It is almost exclusively used for virgin canes at planting or when they are 5-6 months old. The ashes from the bagasse furnace are mixed with press cake or molasses or even with bagasse to form a compost which has been called 'Saccharogene'. Bagasse which is in the form of a liquid has also been tried on the soil mixed with other substances, but it is stated that it remains in an unchanged state for a considerable time.

Filter press cake is a valuable manurial material containing phosphorus, calcium, phosphate, nitrogen and organic matter. Unfortunately, detailed analyses of Indian press-muds are not available, but analysis of a few samples has shown that they differ in composition from those of other countries. Table I gives a fair idea of the composition (on a dry basis) of press cake from Indian sulphitation factories, although there will be considerable variation between the products of different factories and between the products of sulphitation and of carbonatation factories, the latter containing far more mineral matter than the former. For purposes of comparison, average composition of the material in Hawaii and Mauritius is also given.

TABLE I
Composition of press cake from Indian sulphitation factories
(Dry basis)

Constituent	Raval-gaon sugar factory	Experimental sugar factory	Mansurpur sugar factory	Another factory	Hawaii	Mauritius
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
Organic matter	74.0	67.0	..	63.0
Nitrogen	1.47	1.0	1.43	1.0	1.85	..
P ₂ O ₅	4.4	4.2	..	5.0	8.9	..
CaO	10.6	9.8	..	10.0	11.3	..
K ₂ O	..	2.3	..	7.0	0.4	..

Press cake can be used for the benefit of the land either by itself or in admixture with other waste materials. It may also be applied after being mixed with artificial fertilizers like ammonium sulphate or with ferrous sulphate. The mixtures at present supplied to the ryots by some Departments of Agriculture. When used by itself directly it can be ploughed into the fields or allowed to decompose in a heap and then spread on the land. When used along with other agricultural waste materials, it is preferable to prepare a compost which will convert the organic matter in it and in the other materials to a form in which they can be readily assimilated by the crop. The easily available product which suggests itself is cane trash or in some cases, bagasse. Such vegetable refuse as is available should also be used. In most of the factories cane is supplied in bullock carts and cattle urine and cowdung are available and can be used as starters. In addition, diluted molasses and effluent s

serve the same purpose, effluent water being used for preparing cowdung.

The use of press-mud mixed with other waste material is to utilize waste products and also to obtain an increased yield of manure giving larger quantities of humus.

Different processes of composting have been developed and the methods more or less been standardized; for new materials, however, experiments have to be conducted to determine the period of composting, composition of compost and the most suitable process. The well-known process is the *Process* and various modifications suitable for application to different materials have been evolved. In the case of sugar factory products, over the process of composting adopted, either the raw materials or the end products have to be stored for 4-6 months if they have to be applied to land at the time of planting or a little earlier. For application to the crop, this long storage may not be necessary, as the programme of work is so arranged as to have the compost ready just when it is required. It is, therefore, considered necessary to investigate thoroughly all the possible methods of using filter press cake, cane-trash and bagasse before recommending to the factory owners and cultivators the best method of utilizing for the benefit of the land.

EXPERIMENTAL

Composition of the materials used

Filter press cake (sulphitation) contained moisture, 62.6 per cent. The cake after drying in the sun for 15 days in a thin layer on the ground contained 10 per cent moisture, 1.38 per cent nitrogen and 40 per cent carbon.

Sun-dried cane trash contained moisture 7 per cent; N, 0.24 per cent; C, 40 per cent (on dry basis). Partially dried bagasse contained 30 per cent moisture, 0.14 per cent N and 40 per cent C (on dry basis).

Sugar factory molasses contained 0.27 per cent N and 24.0 per cent C. Cowdung had 61.5 per cent moisture, the nitrogen and the carbon in the oven-dried sample being 0.92 per cent and 14.0 per cent respectively.

Composting

For the preparation of the heaps, sun-dried press-mud (sulphitation), cane-trash cut into lengths of 2-3 in. and small lengths of bagasse well-dried in the sun for 8-10 days were used. The requisite quantities of materials for a total weight of each heap was 8 cwt. the proportion of the ingredients being in different cases, as given in Table II) were mixed together thoroughly with the activators and made into a heap of suitable dimensions. Temperature of the heap was noted periodically to have an idea of the progress of decomposition. The maximum temperature attained in most heaps was about

A) The heaps were subjected to three turnings, once after 15 days, again after another 15 days and finally a month later, with the addition of a thin layer of molasses and cowdung prepared with effluent water. Water was added occasionally if the heaps became too dry. Composts were taken out ready when the heaps had developed a crumbled powdery structure with a grayish black appearance. Nitrogen and moisture were determined at this stage. Ten heaps (experiments 1-10) of different compositions were prepared in duplicate and composted by this method.

TABLE II

Loss of nitrogen and dry matter from heaps of different composition

Expt.	Composition of the heap						No. of turnings	Time of composting in months	Percent- age loss of dry matter	N per- centage in the compost (on dry basis)	P
	Press- mud	Cane- trash	Bag- asse	Molasses and cawdung per cent of each on the wt. of heap	C : N ratio	N per cent					
1A XX .	1	1	...	2 per cent	41 : 1	0.83	3	5	49.6	1.43	
1B .	1	1	...	"	41 : 1	0.83	3	6	53.7	1.42	
2A .	3	1	...	"	33 : 1	1.1	3	6	61.0	1.69	
2B .	3	1	...	"	33 : 1	1.1	3	6	60.0	1.35	
3A .	2	1	1	"	42 : 1	0.79	3	6.6	64.5	1.33	
3B .	2	1	1	"	42 : 1	0.79	3	6.6	63.2	1.30	
4A .	1	...	1	"	52 : 1	0.76	3	7	63.0	1.17	
4B .	1	...	1	"	52 : 1	0.76	3	7	61.4	1.35	
5A .	3	...	1	"	37 : 1	1.06	3+1	6.8	59.7	1.56	
6B .	3	...	1	"	37 : 1	1.06	3	6	62.6	1.60	
6A X .	1	1	...	"	20 : 1	1.66	3+1	6.3	67.0	1.83	
6B X .	1	1	...	"	20 : 1	1.66	3	6	63.1	1.88	
7A X .	3	1	...	"	23 : 1	1.66	3+1	4.5	39.4	1.75	
7B X .	3	1	...	"	23 : 1	1.66	3+1	4.6	44.9	1.86	
8A X .	2	1	1	"	22 : 1	1.64	3+1	4.8	50.7	1.83	
8B X .	2	1	1	"	22 : 1	1.64	3+1	4.8	46.1	1.77	
9A X .	1	...	1	"	23 : 1	1.63	3+1	4.7	50.0	1.71	
9B X .	1	...	1	"	23 : 1	1.63	3+1	4.7	51.2	1.58	
10A X .	3	...	1	"	23 : 1	1.66	3+1	4.7	54.4	1.88	
10B X .	3	...	1	"	23 : 1	1.66	3+1	4.9	55.2	1.88	
11A .	1	1	...	1 per cent	41 : 1	0.83	90 (daily)	5.5	62.2	1.32	
11B .	1	1	...	"	41 : 1	0.83	90 (daily)	5.5	62.0	1.34	
12A .	1	1	...	2 per cent	41 : 1	0.83	17 (daily)	4.6	55.0	1.29	
12B .	1	1	...	"	41 : 1	0.83	17 (weekly)	4.5	45.7	1.12	
13A .	3	1	...	"	33 : 1	1.1	17 (weekly)	4.5	53.0	1.44	
13B .	3	1	...	"	33 : 1	1.1	17 (weekly)	4.5	53.2	1.46	
14A .	1	1 per cent	29 : 1	1.38	11 (weekly)	4	54.4	1.55	
14B .	1	"	29 : 1	1.38	11 (weekly)	4	50.0	1.41	
15A .	1	...	1	2 per cent	52 : 1	0.80	None	12	30.3	0.89	
15B .	1	...	1	"	52 : 1	0.80	Do.	12	31.7	0.91	
16A .	1	...	1	"	52 : 1	0.80	Do.	12	38.4	0.91	
16B .	1	...	1	"	52 : 1	0.80	Do.	12	40.7	0.93	

X Ammonium sulphate added to the heaps to raise the percentage of nitrogen

XX P₂O₅ per cent in the compost : 3.3K₂O per cent in the compost : 0.86

- (C) Experiment 11.—Heaps were subjected to daily turning for a period of 6 months. Cowdung and molasses were added at each turning.
- (D) Experiments 12-14.—Turning was done every week with addition of cowdung and molasses.
- (E) Experiment 15.—Press-mud (4 cwt) and bagasse (4 cwt) were added in alternate layers of about 2-3 in. thickness each and the heap was built up of three such layers. Each of the layers was well mixed with a quantity of cowdung and molasses before the next upper layer was placed on it. The layers were not mixed and no turning was given.
- (F) Experiment 16.—Press-mud (4 cwt) and bagasse (4 cwt) were well mixed with a thin slurry of cowdung and molasses and a heap of suitable dimensions was prepared. No turning was given.

RESULTS

A considerable heat was developed in every one of these heaps within a day of preparation, the temperature inside the heaps after a week or ten days rising to 60-65°C. in many cases. After a lapse of 3-4 weeks, fermentation subsided and little heat was developed. Table II contains the results obtained so far. The data obtained show clearly that valuable manure can be prepared from what are considered at present as 'waste products'. Attempts were also made to use press-mud of carbonatation factories in the preparation of composts. These did not prove satisfactory because this mud contains a large proportion of inorganic matter. It contains only 0.5 per cent C and 0.6 per cent N. The addition of organic waste to balance the inorganic matter would reduce the percentage of nitrogen, which is already low.

For adoption by the factories, the method of composting recommended should be inexpensive. Our experiments have only this object in view. Method (B) is obviously unsuitable as labour required will be very large. The most promising methods for adoption on a large scale are (D) and (E). They involve little expense and the longer period of composting is not a disadvantage, as there is a good margin between the close of one crushing season and the next planting season.

As is observed from Table II that in all these methods, there is a loss of weight varying from 20-50 per cent and of dry matter, 40-60 per cent depending on the composition of the heap. Excellent results have been claimed for the fermentation method of composting by Acharya *et al.* [1939]. In this method the heaps are subjected to both aerobic and anaerobic fermentation, but only for a short period in the beginning. Composting of the sugar waste products by this method is being examined, so that the losses can be reduced if possible.

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REFERENCE

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THE DISPOSAL OF POONA SEWAGE FOR IRRIGATION AND CROPPING

BY

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(With Plates V and VI and two text-figures)

ONE of the most important questions in India is maintaining the fertility of the soil, specially in intensively cultivated areas. Every intelligent cultivator utilizes all his farm wastes by returning as much of it as possible to the soil to secure good results but the dearth of bulky manure is always keenly felt in intensively irrigated areas. Villagers round about the towns use refuse and night-soil; green manuring is also resorted to when irrigation facilities are available. In large towns, the removal of excreta by basket and carts is practised for economic reasons and is being gradually replaced by a drainage system for sanitary reasons wherever possible.

From the analysis of effluent received from the Poona city sewage treatment works, it was found that the nitrogen contained in it is roughly equal to 3 lb. per acre per head of population. The money value of these 3 lb. of N is equal to Rs. 1.50 on the basis of market rates and of N contents in oil-cakes, ammonium sulphate and other manures. If such valuable N is not utilized for irrigation but wasted elsewhere, the loss to national wealth due to such waste would amount to lakhs per annum for Poona alone.

METHOD OF DISPOSAL OF CITY SEWAGE

Sewage is at present being directly let into sea or large perennially flowing rivers. Where such facilities are not available, it has to be passed through septic tanks for anaerobic treatment and further through rotary filter for aerobic treatment. Recently the sludge portion of the sewage water is utilized for preparation of gas for power purposes. Sometimes effluent is required to be chlorinated or treated with flocculents. It is only then that it can be let into a *nalla* without any danger to public health. There is another method of utilizing the sewage, and this is by irrigation to crops. This latter method is described in the paragraphs to follow.

POONA SEWAGE DRAINAGE AND IRRIGATION SCHEME

This scheme comprises (a) drainage from the Poona city, cantonment and suburban municipality, etc. The present volume of sewage received per day is about 35 gallons. (b) All this raw sewage is thus collected by gravity to a central pumping station at Bahiroba where this passes through a screen and grit chambers for removal of *katchra* and sand. About 1,000 lbs. of sand are recovered annually. The screened sewage passes then through the balancing tanks; each tank is 250 ft. long and 36 ft. wide. The capacity of each of the tank compartments is about 0.6 million gallons making a total of 1.8 million gallons, for the three tanks. Sewage settles in these tanks about four hours before it is pumped to the head of distributary

ation. (c) Sewage is further led on to a sump pit and pumped. The quantity pumped is about 7 million gallons per day at present. The static lift of high sewage is pumped is 88 ft. from suction level to delivery end.

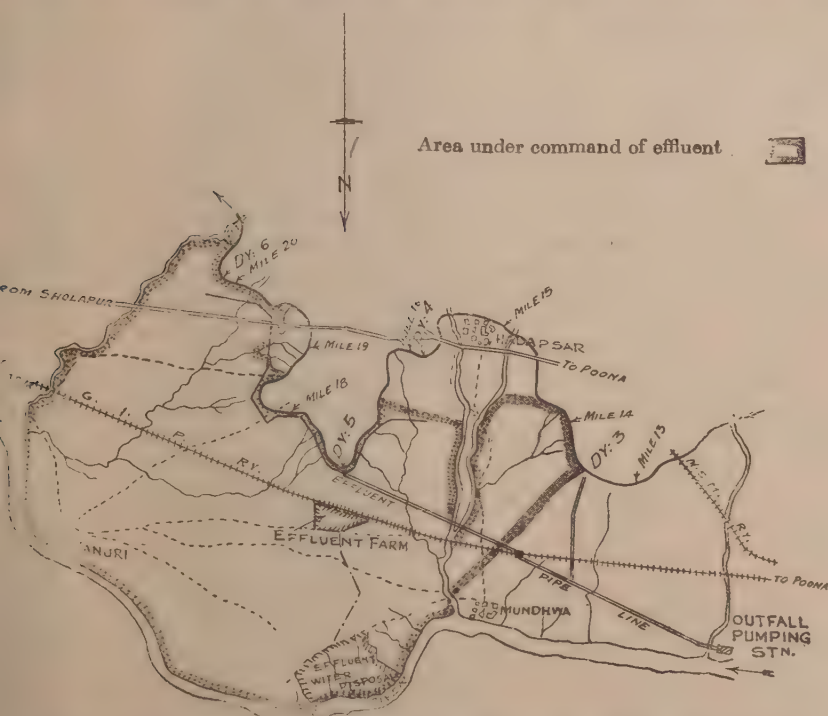
The pumping set consists of four units, one electric unit and three oil units. The three oil units consist of Diesel engines, two cycle vertical type 50 B. H. P. running at 300 revolutions per minute.

Sewage which is pumped passes through a rising main of 30 in. diameter and discharges at a distance of $3\frac{1}{2}$ miles at distributary 5 of Mutha Right Bank Canal. There is also a branch pipe connected from the rising main which discharges at distributary 3. This is described in details by Inglis, Collett and Joglekar [1938].

The discharges are received in the masonry chambers at the distributary and are either diluted, if required, with canal water which is close by or allowed to pass in the raw form for irrigation purposes. Fig. 1 explains the lay-out clearly.

EFFLUENT IRRIGATION AREA

The area which receives sewage irrigation is called effluent zone which consists of areas under distributaries 3, 4, 5 and 6 of Mutha R. B. Canal (Fig. 1). The total area suitable for sugarcane cultivation is about 3,000 acres, but



1. Index plan showing effluent zone, Mutha Right Bank Canal, from mile No. 13 to 20 (scale 1 in. = $\frac{3}{2}$ mile)

only 800—1,000 acres at the most are under sugarcane at present, while the sewage received from Poona is sufficient for supplying N requirements about 2,000 acres on the basis of 300 lb. of N which has been found to be adequate for producing a good crop of sugarcane. As only half the area is therefore normally under sugarcane, Government have acquired an area for disposing of surplus effluent when it is not required for sugarcane. This is done during the latter half of *rabi* season as it is beneficial to cut off sewage at least two months prior to harvesting of sugarcane.

ANNUAL DISCHARGES OF SEWAGE AND ITS MANURIAL VALUE

Table I gives the discharge of sewage received during the preceding five years.

TABLE I

Discharge of sewage, total nitrogen and area irrigated in the zone

Year	Discharge of sewage in c.ft. per second	Discharge utilized in cusecs	Discharge surplussed in winter disposal area cusecs	Total N parts per 100,000	Area sufficient for cane on 300 lb. N basis	Actual perennial area irrigated	Remarks
1935-36 . . .	10.23	9.82	0.41	3.53	Acres 1880	Acres Gunthas 999—30	One cu. ft. for hour—22,500 gallons
1936-37 . . .	9.91	9.55	0.36	3.55	1787	1270—0	
1937-38 . . .	8.55	8.15	0.40	3.79	1620	367—20	
1938-39 . . .	10.43	9.46	0.97	3.50	1830	888—0	
1939-40 . . .	10.77	10.77	...	3.31	1782	1005—20	
Average	1780	906—0	

There is a gradual fall in the total N contents from 1937-38 which is due to increased usage of water in the city. The current year shows highest discharge and is naturally accompanied by an appreciable decrease in the total N contents. The average of five years figures shows that the area for which sewage was sufficient at 300 lb. per acre was about 1,780 acres, whereas an area of 906 acres only has been irrigated.

The average of weekly results of free ammonia, albuminoid ammonia, suspended solids and other useful ingredients during the year 1939-40 are given in Table II.

TABLE II

Manurial ingredients in Poona sewage during 1939-40

(Parts per 100,000)

Season	Free and saline ammonia	Albuminoid ammonia	Total N	Potash as K_2O	Phosphoric acid as P_2O_5	Calcium as calcium oxide	Suspended solids
Hot weather . . .	2.51	0.92	3.43	0.521	1.35	14.287	44.10
Monsoon . . .	2.36	0.79	3.15	0.377	1.737	13.844	44.10
Rabi . . .	2.41	0.84	3.25	0.396	1.969	14.008	53.30

The average discharge was 10.77 cusecs while the suspended solids on average were about 47 parts per 100,000. This would work out to 5,040 or 10,000 carts. Assuming that half of this settles in the water channels, it can be safely said that each acre of the 1,000 acres of cane irrigated in all on an average 5 cart-loads of this matter in addition to the nitrogen gained in sewage. It will be seen from Table II that the Poona sewage as well contains an appreciable amount of potash and phosphoric acid and hence is a complete manure. These figures, however, represent the total potash and phosphoric acid and not necessarily the quantity available. The latter is under further investigation.

It may be noted in this connection that the experiments at the Padegaon Research Station proved that the addition of P_2O_5 to the extent of 100 lb. in addition to the usual standard dose of N top dressing, with sunn-hemp before sowing, gave results which increased the tonnage by 19 per cent accompanied by a 100 per cent *gul* to cane recovery.

At present sewage being in excess of the requirements, each acre of cane receives about 600 lb. N or nearly double the quantity required. No evil effects of these heavy doses on the quality of *gul* have, however, been noticed.

It will be seen that the effluent zone of Poona is a big estate growing about 100 acres of sugarcane and other two seasonal garden crops and perennial trees, vegetables and fruits. These are freely sold in the market with the usual precautions of washing in case of the former and no harmful results have been observed. Sugarcane grown under sewage irrigation is used in Poona for Bombay, both for chewing and for sugarcane juice as a sweet drink.

As N in sewage is in liquid and easily assimilable form and the total available N is applied in many doses, crops are more benefited than was when manure, either farmyard manure or concentrated manure, was applied in the usual way. In the latter case some of the manure is unutilized which is evident from the residual effect of manure on succeeding crops.

Crops irrigated with sewage exhibit a deeper green colour than those irrigated with canal water.

EXPERIMENTS AT EFFLUENT FARMS*

Sugarcane is a principal crop on this side. Several experiments with other crops were tried at the Effluent Farm, Hadapsar, in medium black soil 12-18 in. deep overlying murrum and described by Inglis [1927]. Dr Basu surveyed the soil of this area and has classified this soil as G type† according to the pedic soil classification described in details by Basu and Sirur [1938].

The rotation followed is biennial, i.e. cane and *dhaincha* (*Sesbania aculeata*), green-manure crop, are grown alternately. Sugarcane receives sewage

*Up to 1929 the sewage received from Poona was treated and the effluent obtained therefrom was utilized for irrigation at Hadapsar and hence the Farm is known as Effluent Farm. Since 1930 the sewage is passed directly on to the land. However, the title remains unchanged and the farm is still known as the Effluent Farm.

† Brown-coloured soil 15-24 in. deep over lying murrum (decomposed trap) consisting of about 50 per cent clay and 10-15 per cent silt, pH values near about 8.0, containing moderate quantities of humus and nitrogen. There is low CaO/MgO ratio indicating a general inferior drainage condition of the soil.

throughout, while *dhaincha* requires only one irrigation. Thus out of months, the land receives sewage irrigation for 16 months.

VARIETIES UNDER SEWAGE IRRIGATION

Seven important varieties of sugarcane including Pundia were tried. They were all planted in January. In one series, normal dose of effluent (300 lb.) supplemented by canal water was given, while in the other sewage irrigation was continued till harvest. The experiment was in replicates. Harvesting was done by February next year, after $13\frac{1}{2}$ months. The out-turns are given in Table III.

TABLE III
Performance of different varieties

Name of variety	Normal dose (300 lb. N)				Sewage irrigation throughout (roughly 900 lb. N)			
	Out-turn of cane in tons	Out-turn of <i>gul</i> in tons	Brix	Purity (per cent)	Out-turn of cane in tons	Out-turn of <i>gul</i> in tons	Brix	Purity (per cent)
Pundia	37.85	4.37	18.6	81.5	36.31	3.94	17.9	76.0
Co 417	50.74	6.06	19.0	81.6	50.96	5.66	18.7	79.0
Co 408	53.91	6.17	20.3	80.1	47.12	5.34	20.3	81.0
Co 419	49.87	5.95	21.2	83.7	52.25	6.44	21.4	85.0
POJ 2878	47.35	5.14	19.2	82.0	45.80	5.39	20.4	88.0
Co 411	49.87	5.07	18.9	81.1	47.28	5.27	18.8	81.0
POJ 2883	40.17	4.98	20.8	86.9	41.74	4.95	20.9	87.0

The results of *gul* weights show that Co 419 and POJ 2878 are benefited by continued effluent. Co 411 also shows a small increase. There is a depressing effect of continuing effluent till harvest in the case of Co 417, Co 408, POJ 2883 and Pundia (local cane). Further experiments with different suitable varieties are in progress. Plate V, fig. 1 shows the growth of important varieties.

Out of the above, POJ 2878 and Co 419 were further tested under heavy dose experiments in the following year on plots Nos. 111—116 of the Effluent Farm. These varieties were planted in December and harvested after 12 months on maturity, which was noted by taking brix readings at intervals. The average out-turns from four replicates are given in Table IV.

TABLE IV
Comparative out-turns of Co 419 and POJ 2878 varieties under heavy doses of

Serial No.	Description of experiment	Name of variety	Cane wt in tons per acre	<i>Gul</i> wt in tons per acre	Brix	Per cent of <i>gul</i> to cane	Remarks
A	300 lb. of N, i.e. normal dose	POJ 2878	51.44	5.79	18.35	11.25	
		Co 419	57.92	6.62	20.31	11.40	
B	*900 lb. of N, i.e. crop raised on sewage irrigation alone	POJ 2878	52.66	5.91	18.31	11.23	
		Co 419	60.60	6.86	19.23	11.32	

* The experiments on seeing the effect of sewage irrigation alone on crop and soil were mainly due to Mr U. N. Mahida, B.E., I.S.E., now Deputy Secretary to the Government of Bombay, P. W. D. His personal observations on this aspect of the problem have greatly stimulated research of much practical importance.



FIG. 1. Growth of important varieties under trial at the Effluent Farm and effluent zone (1. Co 419 heavy dose ; 2. Co 419 normal dose ; 3. EK 28 normal dose of sewage (from cultivator's fields) ; 4. POJ 2878 ; 5. Co 426 ; 6. POJ 2883 ; 7. Pundia (Local cane)



B

A

FIG. 2. Growth under heavy doses of nitrogen : A. 300 lb. of nitrogen and sewage diluted with canal water ; B. 900 lb. of nitrogen with sewage irrigation alone (Date of planting 7-1-1939, variety Co 419, age 11 months)

[Note the loose tilth of the ploughed lands lower down which have been under sewage irrigation for 20 years]



FIG. 1. Cultural treatment
Co 419 variety 12
months old

Left : Earthing up
Right : No earthing up

(Note the poor growth under
'no earthing up')

FIG. 2. Cultural treatment
Co 419 variety 12½
months old (inside
view of sub. plots)

Left : Earthing up
Right : No earthing up

(Note the lodging of 'no-
earthing up')



FIG. 3. Cultural treatment
Co 426 variety 12
months old

Left : Earthing up
Right : No earthing up

(Note the good growth of
treated cane on the
left)

In item A, cane was given 300 lb. of nitrogen in 10 months and was further supplemented by canal water, while in item B sewage irrigation alone was given throughout. Plate V, fig. 2 shows the growth of cane under normal and continuous sewage irrigation. There is a distinct difference in the growth of cane between the two treatments. Treatment B, with a large dose of N and with sewage irrigation alone, gives more vegetative growth than treatment A. The difference in yield between A and B is, however, not appreciable. But the experiment shows that it is possible to raise a sugarcane crop under sewage irrigation alone with proper treatment, as detailed further.

EFFECT OF SEWAGE IRRIGATION ON A RATOON CROP

POJ 2878 sugarcane variety was tried with varying doses of N from 300 to 500 lb. of nitrogen. This was planted as usual in the month of December and harvested in January 1939. Ratoon was kept during the year 1939 and harvested in 1940. The varying doses of N in the form of effluent irrigation were continued to the ratoon cane also. The results are shown in Table V.

TABLE V

Turns of POJ 2878 under ratoon condition with varying doses of nitrogen compared to the out-turns of plant cane

Dose of N in lb. per acre	Out-turn of cane in tons per acre (plant cane)	Out-turn of cane in tons per acre (ratoon)	Out-turn of <i>gul</i> in tons per acre (plant cane)	Out-turn of <i>gul</i> in tons per acre (ratoon)	Recovery of <i>gul</i> to cane (plant cane) per cent	Recovery of <i>gul</i> to cane (ratoon) per cent
300	45.42	35.52	5.35	4.33	11.78	12.15
400	45.50	37.3	5.45	4.40	11.99	11.89
500	45.36	39.56	5.37	4.86	11.60	12.30
600	46.97	39.42	5.33	4.57	11.34	12.02
Sewage irrigation roughly 500 lb. N	47.58	42.51	5.89	4.70	12.12	11.54

The results show increased yield of *gul* with increased doses both in the case of plant cane and ratoon cane except 500 lb. dose in the case of ratoon and 600 lb. dose in the case of plant cane.

It must be noted that except sewage irrigation no other manure was given in the ratoon crop. Great care was taken in doing timely partial and complete topping up operations. The results of ratoon tried with Pundia (local cane) variety 417 and Co 419 are given in Table VI.

TABLE VI

Out-turns of local cane and Co varieties under plant and ratoon condition
(Per acre)

Variety	Description	Ratoon		Plant cane	
		Out-turn of cane in tons	Out-turn of <i>gul</i> in tons	Out-turn of cane in tons	Tons of gur per acre
Pundia	Normal dose 300 lb.	30.60	3.32	..	4.4
Co 417	Do.	40.41	4.64	47.24	4.7
Co 419	Do.	35.03	4.39	48.42	5.1

EFFECT OF SEWAGE IRRIGATION ON *ADSALI* CANE

In this zone, the problem of sewage disposal is of great importance, especially in the months of November—February when plant cane normally does not require sewage. Some of the surplus sewage could be utilized by *adsali* cane planted during monsoon. This experiment was continued with a view to finding out which of the new cane varieties gave best results under this condition. In previous experiments Pundia had failed to grow as an *adsali* cane. Hence four important varieties were tried. Planting was done on 15 June and 1 August and harvesting was done after 18 and 16 months respectively when the crop showed signs of maturity. The out-turn was as shown in Table VII.

TABLE VII

Out-turn of varieties under adsali plantation

(Per acre)

Name of variety	June planting			August planting			June plantation difference from the control	August plantation difference from the control
	Cane weight in tons	No. of canes at harvest	Brix	Cane weight in tons	No. of canes at harvest	Brix		
POJ 2878	70.1	42,225	18.28	42.6	35,131	17.37	2.8	5.6
POJ 2883	67.3	35,400	17.89	37.0	31,781	19.29	Nil	Nil
Co 419	76.8	54,450	15.18	48.9	44,906	17.44	9.5	11.90
Co 411	86.9	48,750	16.11	49.1	36,469	16.23	19.6	12.10
Note.—Significance for both the June and August plantations combined			9.71		Significance figures		7.92	5.77

The experiment was replicated four times. The statistical treatment of the results showed June plantation to be significantly better than August plantation, while considering the two plantations separately Co 419 and Co 411 gave significantly higher yields than the rest.

It will be seen that Co 411 gave the highest yield of cane, about 87 tons in the case of June plantation. There was also profuse tillering and higher

ber of canes were recorded in the case of Co 419 variety. These results show that Co varieties are more suited for planting in June and August than other varieties. Of the two, plantation in June is much superior to August plantation from the point of view of growth.

Early cane plantation

Sugarcane varieties were also planted in October. It was found that Co 2878 and Co 419 gave respectively 3 and 4 tons of cane more than the control variety planted under similar soil conditions.

CULTURAL TREATMENT UNDER SEWAGE IRRIGATION

Under the conditions prevailing in the effluent zone, the necessity of early cultural operations cannot be over-emphasized. The most important of these operations are the partial earthing up and earthing up. The first operation removes unnecessary rootlets (*jarwa*), loosens the top soil and gives better ventilation. It also brings some fresh soil from the ridge nearer the root zone. Irrigation is given five to six days after this operation. Good results are seen a month after this operation. The next operation of earthing up is done when the canes show two or three well-developed nodes. During this operation the soil is loosened by pickaxes which cuts off the unnecessary old roots and rootlets while the loosened fresh soil from the ridge is shifted to the cane rows in the furrows. Thus, after this operation, the cane is on the edge. This operation is started two days after the sewage irrigation is given which is followed by a light sewage irrigation (called *bore padne*) after four days. This latter irrigation can be omitted if there are rains. This operation greatly stimulates the crop later. It is shown by Rege and Wagle [1939] that this operation can be omitted for a sugarcane crop when the tonnage is about 40 or so. To test if this operation can as well be recommended for omission by sugarcane growers in the effluent zone, a systematic experiment was carried out with important varieties and the results are shown in Table VIII.

TABLE VIII

Out-turn of Co 419 and Co 426 cane varieties under cultural treatment

Treatment	Co 419 variety cane yield		Co 426 variety cane yield		Co 419 difference from the control	Co 426 difference from the control
	In tons	Brix	In tons	Brix		
Partial earthing up and earthing up	47.57	21.12	42.70	18.82	12.59	10.9
No partial earthing up but only earthing up	47.03	20.63	44.50	18.83	12.05	12.6
Partial earthing up but no earthing up	36.27	19.78	34.90	16.79	1.29	3.0
No partial earthing up. No earthing up	34.98	20.25	31.90	17.87	Nil	Nil
Significance figures Co 419	7.71	..
Significance figures Co 426	9.09

These results show the great influence of earthing up on yield and also brix. Plate VI, fig. 1 (front view of the experiment) shows the difference growth between earthing up and non-earthing up, fig. 2 shows the head lodging caused due to non-earthing up even for a 40-ton crop and fig. 3 is Co 426 variety under similar treatments. These results clearly show that under the conditions prevailing in this zone this important operation has to be done regularly to ensure a good crop. This system is closely followed by the irrigators on the Mutha Canals, specially of this zone, under the supervision of the staff of the Effluent Farm.

FODDER CROPS

Regular experiments were laid out in the same type of soil (G type) to show the out-turns of different fodder crops under sewage irrigation.

The out-turns are given in Table IX.

TABLE IX
Out-turn of different fodder crops per acre

Name of crop	Canal irrigation under standard manure dose (lb.)	Sewage irrigation only (lb.)	Remarks
<i>Hundi jowar</i> * . . .	12,480	33,561	Hot weather fodder
<i>Nihwa jowar</i> * . . .	27,736	Nil	Monsoon fodder
Maize	15,000	33,000	
Berseem	29,520	31,160	Considering six cuttings
Lucerne	26,040	54,347	For one year

**Andropogon sorghum*

The out-turn under sewage irrigation is more than double the out-turn under canal irrigation.

EFFECT OF SEWAGE IRRIGATION ON SOIL

The object was to see if any deterioration in soil tilth was noticeable under continuous sewage irrigation. For this purpose, careful selection was made of soils in the effluent zone, irrigated by canal water and under continuous sewage irrigation for 20 years. Fig. 2 shows the exact position of profiles examined. In all five comparisons were made. The results are given in Table X.

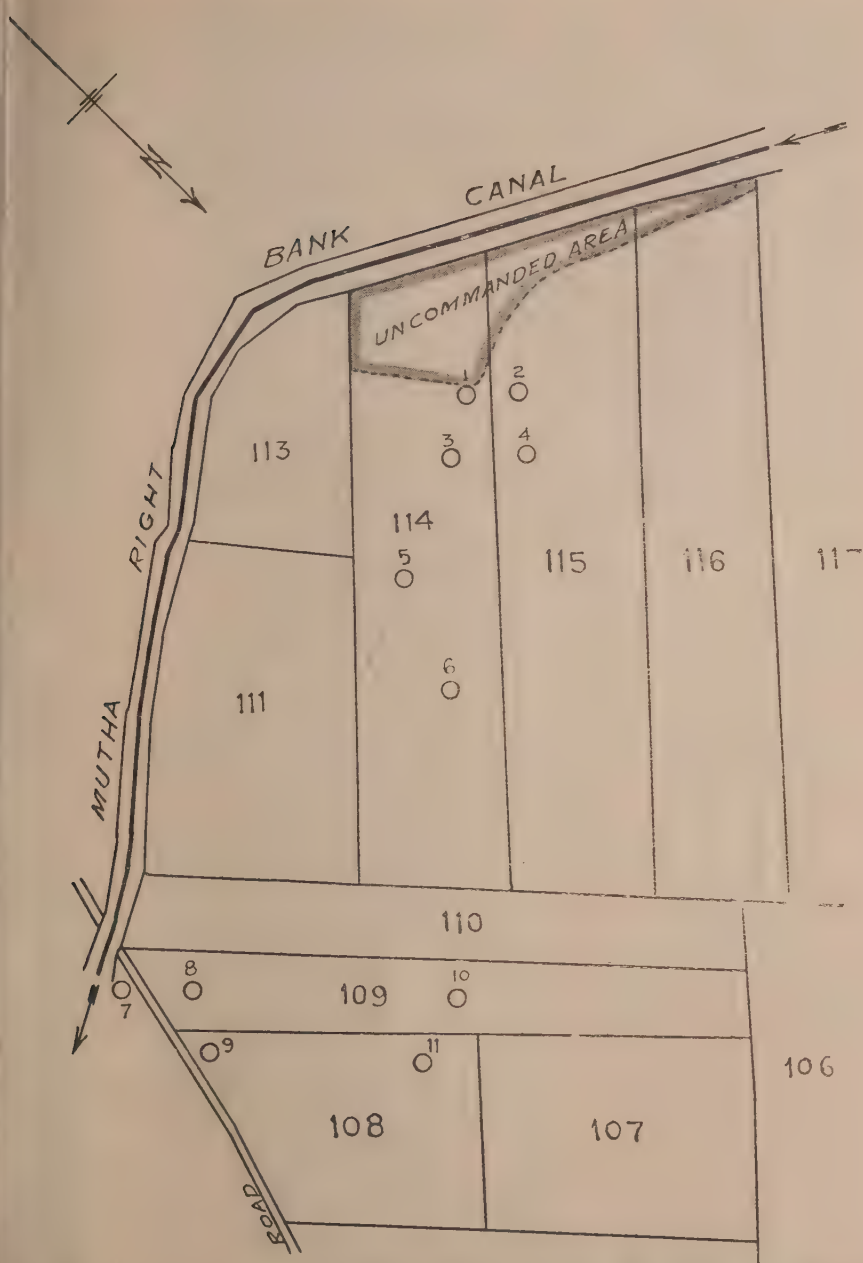


FIG. 2. Plan showing positions of profiles where soil samples were taken for comparative study of soil under and outside sewage irrigation (scale 1 in. = 660 ft.) Profiles Nos. 1, 3, 5, 8 and 10 are under canal irrigation and Nos. 2, 4, 6, 9, 11 and under continuous sewage irrigation; profile No. 7 under dry crops only

TABLE X
Results of soil tests under sewage irrigation and canal irrigation alone

Profile No.	Soil depth (in.)	Canal irrigation						Sewage irrigation					
		Capillary rise in 300 minutes (in.)			pH values in			Capillary rise in 300 minutes (in.)			pH values in		
		In water	In N NaCl solution	Distilled water	N KCl solution	Per cent CaCO ₃	Per cent humus	Per cent soluble salts	In water	In N NaCl solution	Distilled water	N KCl solution	Per cent CaCO ₃
I	0-6	2.55	3.25	8.24	6.71	2.8	0.597	0.075	2.55	2.40	7.71	7.18	3.20
	6-12	1.85	2.20	7.98	6.94	2.75	0.944	0.075	1.00	2.15	7.94	7.01	3.85
II	0-6	2.10	2.50	7.52	7.18	3.20	1.08	0.10	1.00	1.55	7.84	7.80	3.05
	6-12	1.55	1.40	7.58	7.18	3.20	1.82	0.125	1.55	1.55	8.32	7.46	3.80
III	12-24	1.05	1.35	8.00	6.60	3.25	...	0.15	1.30	1.25	8.32	7.36	4.42
	0-6	1.50	1.15	7.51	6.24	2.90	0.609	0.075	2.15	2.05	7.45	7.57	2.85
IV	6-12	1.10	1.22	7.54	6.34	2.82	0.81	0.125	2.50	1.80	7.58	7.14	3.50
	0-6	1.30	1.65	7.82	7.07	3.35	0.98	0.15	1.45	1.40	7.58	7.84	3.20
V	6-12	1.20	1.45	7.82	6.82	3.10	0.96	0.10	1.15	1.05	7.57	7.06	3.07
	12-24	1.30	1.60	7.92	7.42	4.67	0.79	0.15	2.15	2.10	7.32	7.62	2.97
VI	0-6	1.70	1.75	8.24	7.44	4.75	1.46	0.17	1.00	1.05	7.32	7.57	3.30
	6-12	2.95	3.90	8.24	7.30	3.87	0.62	0.16	6.0	6.0	7.81	7.32	4.75
VI	0-6	1.15	1.80	8.24	7.25	2.85	1.03	0.10	} Profile never under any irrigation but under cropping				1.24
	6-12	1.35	1.65	8.08	7.14	2.75	0.72	0.10					1.46

NOTE.—Humus was estimated by Sigmund's method modified in this Laboratory. Soluble salts were estimated by Dionic water tester

The capillary rise tests in canal water and in N NaCl solution for profiles of canal and sewage irrigation show decidedly less difference between the readings in the case of effluent irrigation. In certain cases, the capillary rise with N NaCl is slightly less than with ordinary water which show considerable soil improvement under sewage irrigation. The pH values were found by antimony electrode [Puri, 1932] in distilled water and in N KCl solution and gave similar indications. The pH values are decidedly low in the case of sewage irrigated profiles, while the difference between distilled water and N KCl is small as compared to canal water: this shows comparative enrichment under canal irrigation. The per cent calcium carbonate throughout the profile vary from 2.5 to about 4.5 with slightly more calcium carbonate in sewage-irrigated profiles. The humus contents are also slightly more in sewage-irrigated soils as compared to canal-irrigated soils under similar conditions. The total soluble salts show a little increase under sewage irrigation as compared to canal-irrigated soils but this quantity is as good as we find in normal soils of the type.

The exchangeable bases were found out by method advocated by Puri [1. 2]. Replaceable calcium was also found out by improved acetate method as advocated by him. The results are given in Table XI.

TABLE XI

Exchangeable bases under canal irrigation and sewage irrigation (m. e. per cent)

Soil depth (in.)	Under canal irrigation				Under sewage irrigation			
	Replaceable Na plus K	Replaceable Mg	Replaceable calcium	Total replaceable bases	Replaceable Na plus K	Replaceable Mg	Replaceable calcium	Total replaceable bases
0-6	0.53	6.66	38.5	45.69	0.44	5.32	39.0	44.76
6-12	0.53	7.82	39.5	47.85	0.35	6.18	43.30	49.84
0-6	0.89	6.86	41.0	48.75	0.53	5.52	46.30	52.35
6-12	0.80	7.24	40.0	48.04	0.26	5.32	46.50	52.18
12-24	0.98	6.66	40.5	48.14	0.44	4.94	45.50	50.88
0-6	1.068	8.48	37.0	46.55	0.56	9.24	37.0	46.804
6-12	0.89	8.96	38.5	48.35	0.66	8.00	37.5	46.16
0-6	1.07	9.16	39.0	49.23	0.66	7.62	42.50	50.78
6-12	0.98	8.58	39.0	48.56	0.94	9.60	43.50	54.04
12-24	0.89	8.88	37.0	46.77	1.028	10.28	45.0	56.30
0-6	1.07	5.70	42.0	48.77	1.50	7.80	42.50	51.81
6-12	0.62	4.94	37.5	43.06	0.66	6.40	39.50	46.56
0-6	1.51	8.30	33.0	42.81	Never under any irrigation but under dry crops			
6-12	1.33	5.44	32.5	39.27				

These results show that out of 12 cases, 10 show a gain in exchangeable bases in the case of sewage-irrigated soils. It also shows an increase in replaceable

calcium. The percentage of monovalent bases are very low in both, and specially under sewage irrigated soils. These results indicate that calcium from sewage water (Table II) takes part in exchange phenomena and more calcium is made available under sewage irrigation. This point is under further investigation. However the results given do show that the soils in the effluent zone have not deteriorated by sewage irrigation as is commonly believed.

Miscellaneous

In this zone, as the original potable water supply from wells, etc. were danger of contamination due to the effluent irrigation, adequate piped water supply arrangements have been made for drinking purposes from specially constructed reservoirs for the purpose.

SUMMARY

(1) A detailed description of the disposal of Poona sewage from the city to the pumping station, some three miles away from Poona, and thence to the cultivators' fields is given.

(2) The composition of Poona sewage is given, showing that it is a valuable and complete manure.

(3) Out-turns of sugarcane varieties, specially under sewage irrigation are given which show that high yields are obtained up to 60 tons of cane per acre in the case of Co 419 variety and up to 50 tons per acre in the case of POJ 2878. The latter (POJ 2878) is a better cane because of early maturity and good quality of *gul*.

(4) (a) Co 411 and Co 419 are very good as plant and *adsali* canes. In *adsali* plantation, cultivators prefer to grow also POJ 2878 and POJ 2883 as a mixture with Coimbatore varieties as it is observed that mixed crushing gives better quality of *gul* than with Coimbatore varieties only.

(b) POJ 2878, Co 417 and Co 419 ratoons well under sewage irrigation.

(5) Necessity of proper cultural operations under sewage irrigation emphasized. Earthing up and partial earthing up operations are essential to maintain suitable soil conditions and get better returns.

(6) The out-turns from cane varieties and other fodder crops under sewage irrigation compare very favourably with the out-turns obtained elsewhere in similar crops under canal irrigation with bulky manures and artificial fertilizers [Vagholkar and Patwardhan, 1940] on sugarcane varietal trial, and of fodder crops as given in *Bulletin* No. 100 of Department of Agriculture, Bombay.

(7) Soils of the type studied do not show any deterioration under continuous sewage irrigation, provided suitable soil conditions are maintained on the other hand some improvement is noticeable which is shown by capillary rise, pH values and exchangeable bases.

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FIXATION OF ATMOSPHERIC NITROGEN IN LIVING FORMS

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INTRODUCTION

THE most striking result of the researches of Pasteur in the last century is perhaps the revelation of a world of microbes incessantly at work taking part in all vital processes in relation to the maintenance of life on this planet. Among the great variety of micro-organisms that are responsible for important processes in the soil, the nitrogen-fixing organisms have formed the subject of fascinating study.

In the middle of the nineteenth century Boussingault suggested that the fertile earth contains certain living organisms some of which take part in the fixation of nitrogen in the soil. Jodin [1862] first demonstrated that nodules growing in nitrogen free medium fixed nitrogen from the atmosphere. A few years later, Berthelot [1885] announced that he obtained increases in the nitrogen content of normal but not sterilized soils. He further showed that the rise in the organic nitrogen content of soils left unactivated for a period of several months is due to the microbial activity. Later, Hellreigel and Wilfarth [1888] discovered the nodule-organisms, *Rhizobium*, in the roots of leguminous plants and showed that these organisms, in association with the leguminous plants, bring about nitrogen fixation.

The above findings were soon followed by increasing evidence to show that nitrogen fixation in the soil was brought about by the activities of the micro-organisms present in the soil. Winogradsky [1893] isolated a new aerobic organism from the soil, *Clostridium pasteurianum*, which was found to fix nitrogen in the deeper layers of the soil. A more important discovery in this direction was that of *Azotobacter chroococcum* and *Azotobacter agilis* by Beijerinck [1901]. These organisms were isolated from soils and canal waters

and found capable of vigorous nitrogen fixation. Thus in the course of few decades the process of nitrogen fixation in the soil by micro-organisms became an established fact.

The most important conclusion respecting biological nitrogen fixation which was arrived at by the beginning of this century, was that there are generally two classes of micro-organisms that fix nitrogen in the soil; the one class, the non-symbiotic or the free living, having the inherent capacity of fixing atmospheric nitrogen in their bodies, and the other class, the symbiotic, working in combination with plants belonging to the natural order Leguminosae. Subsequent researches in this field have shown that the faculty of using molecular nitrogen is also shared by a few other higher forms of life.

The role of micro-organisms in the fixation of nitrogen and its consequent influence on soil fertility and crop production was soon recognized. Since then a large volume of literature has grown up around this subject, but our knowledge of the biochemical changes that lead to the fixation of nitrogen is still meagre. An attempt is made in this paper to discuss the more important work done during the past 40 years on this problem.

NITROGEN FIXATION IN FREE-LIVING BACTERIA

Among the micro-organisms in which the power of fixing nitrogen is manifested, the free living bacteria constitute the most important group. They occur generally in soils and grow under the same environmental conditions as the other soil bacteria; but unlike the latter, when an adequate supply of nitrogen is not available in the medium, they have the power of building their body proteins from the nitrogen of the atmosphere. Two distinct groups have been recognized in this class of non-symbiotic bacteria, the aerobic and the anaerobic. *Azotobacter* and *Clostridium* are the typical of these two groups of organisms, which are widely distributed in nature. Owing to the fact that *Azotobacter* can be easily isolated from the soil and that it fixes comparatively large amounts of nitrogen in artificial media, this organism has been studied to the greatest extent.

Occurrence, morphology and structure of *Azotobacter*

Azotobacter is generally present in soils whose pH is not lower than 6 [Gainly, 1925] and is occasionally found to occur in the ocean and marine fresh waters along with algae and other plankton organisms [Keutner, 1900]. Bergey [1930] classifies them into six distinct species, of which four only are frequently met with in the soil, *A. chroococcum*, *A. agile*, *A. vinelandii* and *A. beijerinckii*. He defines them as: 'Relatively large rods or even cocci, sometimes almost yeast-like in appearance, dependent upon the oxidation of carbohydrates. Motile or non-motile. When motile, with a single or a tuft of polar flagella. Obligate aerobes usually growing in a film upon the surface of the culture medium. Capable of fixing atmospheric nitrogen when grown in solutions containing carbohydrates and deficient in combined nitrogen'.

A medium containing dipotassium hydrogen phosphate (0.2 gm.), magnesium sulphate (0.2 gm.), sodium chloride (0.2 gm.), calcium sulphate (0.1 gm.), ferrous sulphate (0.01 gm.), sodium molybdate (0.05 gm.) and manganese sulphate (0.05 gm.) along with 5 gm. of calcium carbonate and 10 gm. of sugar in a litre of media is used for isolation and study of the bacteria.

The cells of the different species vary in size, shape and other morphological characteristics. Bonazzi [1915] has found that *Azotobacter* cells are of a complex nature and different stadia of cytological make-up. The granules are of metachromatic nature and seem to have no relation to the function of the cell. Lewis [1937] has distinguished volutin bodies, fat granules, metachromatic granules inside the cell. He has also shown that the organism is composed of gum. Lohnis and Smith [1913, 1923] have suggested a life-cycle in its mode of reproduction, but adequate evidence on this point is still wanting. This organism differs from the schizomycetes in its morphological and cultural characteristics but is closely related to yeasts (Lohnis, 1933; Riccardo, 1925). The role of the various cell constituents, more particularly the characteristic cell inclusions, in nitrogen fixation is not well understood. From an interesting biological study, using the respirometric technique, Iwaski [1930] has shown that on nitrogen-free medium cell multiplication may occur without nitrogen fixation, and conversely nitrogen fixation without cell multiplication. Whatever may be the underlying causes, the dissociation of nitrogen fixation from cell multiplication and the possibility of obtaining storage of nitrogen compounds accompanied by increase in cell size without increase in numbers is an interesting phenomenon before demonstrated. He has also distinguished three different phases: multiplication phase, fixation phase and storage phase as its cultural characteristics.

Metabolism in Azotobacter

(i) *Nutritional requirements.*—The food requirements of this organism consist of a source of energy, supply of oxygen, water and certain minerals. A variety of carbon compounds, particularly the sugars, can serve as energy source for this organism. However, it is generally found that mannite is not efficiently utilized for nitrogen fixation. Conflicting views are held regarding the relative nitrogen-fixing efficiencies of other carbohydrates (Rimskii, 1908; Stranak, 1908). The salts of a wide range of organic acids are also used as an energy source by this bacteria [Guittonneau and Chevalier, 1936] and among these the sodium salts of lactic and benzoic acids are found to be better energy sources for fixing nitrogen. There was nearly a constant relation between the amount of nitrogen fixed and the heat of combustion of fatty acids. Kuba [1930] has reported that the fatty acids of even number of carbon atoms are more useful for nitrogen fixation than the odd ones. At concentrations of the order of 0.05 per cent phenol in the medium, the organism fixes 9-11 mg. of nitrogen per gm. of phenol oxidized [Guittonneau and Chevalier, 1936]. The organism also uses the lower alcohols (for example methyl alcohol) as a source of energy for nitrogen fixation [Mockeridge, 1915]. Some of the typical results obtained by using different carbon compounds are given in Table I [Mockeridge, 1915].

It may be noted that in the above experiments the efficiency of nitrogen fixation is calculated from the amount of nitrogen fixed per gram of energy material added, without taking into consideration the amount of carbon that may have been utilized by the organism. It would therefore be useful to find out the nitrogen-fixing efficiencies of the different carbon sources as a ratio, $\frac{\text{N fixed}}{\text{C utilized}} \times \text{time}$. The probable relationship between this

efficiency and the nature and configuration of the different carbon compounds used may throw light on the role of these compounds in nitrogen fixation.

Ranganathan and Norris [1927] have shown that within certain limits the amount of nitrogen fixed is proportional to the concentration of sugar present in the medium. Bonazzi [1921, 1924] differentiated between 'fermentative power' or first stage in the growth of the organism when nitrogen assimilation is at a maximum and the second or maintenance phase. During the second stage the carbohydrates are actually reworked, partially burnt to liberate energy and partially utilized in the building up of soluble products. During the early periods of growth a unit of cellular substance could utilize in a unit of time 5.45 units of sugar and after an incubation period of 5 days only 0.28 units of sugar. Very small concentrations of sugar of the order of 0.01 per cent are, however, utilized more efficiently for nitrogen fixation than the higher ones [Truffaut and Bezssonov, 1922].

TABLE I

Material	(n) Nitrogen fixed on 1 gm. of energy material (in mg.)	(e) Efficiency of nitrogen fixation (n) time taken in days to com- pletely use 1 gm. substance
Polysaccharides—		
Gum arabic	6.13	0.102
Gum tragacanth	9.13	0.457
Rice starch	6.40	0.355
Dextrin	6.62	0.331
Inulin	9.76	0.610
Sugars—		
Arabinose	9.28	0.309
Xylose	9.08	0.332
Dextrose	6.57	0.329
Levulose	10.32	0.573
Galactose	6.20	0.282
Sucrose	7.28	0.332
Maltose	7.55	0.444
Lactose	3.39	0.099
Alcohols—		
Methyl	2.10	0.050
Ethyl	4.02	0.119
Propyl	9.20	0.417
Isobutyl	4.69	0.065
Ethylene glycol	16.74	0.492
Glycerol	5.00	0.089
Mannitol	11.62	0.726
Acids—		
Malic	5.19	0.325
Tartaric	4.54	0.162
Succinic	8.60	0.430
Malonic	5.32	0.266
Mucic	6.79	0.170
Lactic	12.01	0.706

It is of interest to note that the utilization of energy material in relation to growth and nitrogen fixation by *Azotobacter* is unique. The quantity of nitrogen fixed for every 100 gm. of glucose utilized has not been more than 1 mg. even under the most favourable conditions; whereas under thermodynamically ideal conditions when all the energy of sugar decomposition is used for fixation only 1.34 mg. of glucose is necessary for fixing 1 mg. of nitrogen. Burk has also shown that whether it fixes nitrogen or not, the organism uses the same amount of carbohydrate to produce 1 gm. dry weight of cell substance. He has found that in low oxygen tensions, *Azotobacter* uses the carbohydrates more efficiently for nitrogen fixation. At 0.001 atm. oxygen the organism uses only 1 gm. of sugar to produce 1 gm. dry weight of cell substance, whereas in most aerobic organisms, such as yeast and bacteria, 10 gm. of sugar is used to produce 1 gm. dry weight of cell substance. Perhaps, *Azotobacter* normally uses oxygen greatly in excess of its metabolic needs and its efficiency is therefore raised by decreasing its oxygen consumption and increasing its growth rate. It would appear from the above that the high consumption of a large quantity of carbohydrate occurs during nitrogen fixation, it need not be because of nitrogen fixation. The role of carbohydrate in nitrogen fixation is still awaiting solution.

Celluloses and hemicelluloses are not directly utilized by *Azotobacter* for nitrogen fixation; but these can serve as food material when the organism is grown in association with cellulose-splitting organisms [Koch and Seydel, 1914; McBeth, 1914; Tuorila, 1938; Deehm, 1932; Buckstag, 1936; Hunter, 1930]. Vandecaveye *et al.* [1934] have shown that the soil organic matter is also utilized by this organism for nitrogen fixation. Lipman and De Nijl [1925] have found that the soil solution is more potent than the resi-

Soil humus in small doses exerts a stimulatory influence on the organism for nitrogen fixation [Voicu *et al.*, 1930]. The effect is proportional to the concentration up to a limit, the optimum being 50 p.p.m. Iwaski [1930] and Voicu [1930] have suggested that the stimulation of nitrogen fixation by humus is probably due to the lowering of oxygen tension and consequent slowing up of oxidation and that the effect may be more on assimilation of nitrogen than fixation. Burk *et al.* [1932] explained the effect as due to the presence of iron in a more suitable organic combination in humus.

Itano [1925] and Bottomley [1920] have shown that vitamin B₁ and nucleic acid present in peat stimulate growth and nitrogen fixation by *Azotobacter*; and a number of other workers [Reed and Williams, 1915; Parkeridge, 1915; Hunter, 1922; Guittonneau and Chevalier, 1936] have reported that the presence of chemicals such as allantoin, urea, esculin, quinic acid depress nitrogen fixation.

In addition to energy supply some minerals in optimum concentration are also found to be necessary for the growth of *Azotobacter*, and a few among these are specific for nitrogen fixation. The presence of manganese in available form is found to stimulate nitrogen fixation; Gregario [1916] and Masolana [1938] have found that it accelerates the process in increasing concentrations up to an optimum limit of 0.0006 Mn ion per 100 c. c. when ten times as much nitrogen is fixed as in its absence. Bortels [1930, 1936] and Nilsson [1936] have reported that minute quantities of molybdenum and

vanadium exert a definite influence on nitrogen fixation, probably they act as catalysers. Concentrations of 1 in 50 million molybdenum and 1 in 10 million vanadium increase nitrogen fixation by *Azotobacter* hundredfold and the presence of Wolfram somehow increases their favourable effect. Molybdenum has no influence on the growth of this organism in combination with nitrogen [Burk and Horner, 1936, 2], if N_2 gas is absent from the medium. From this and other physico-chemical evidence adduced by Burk [1934] it is clear that molybdenum (replaceable by vanadium) is specific for nitrogen fixation.

Phosphorus compounds have been found to accelerate the activities of this organism, but the quantities required are very small; 1 p.p.m. is sufficient to permit growth and nitrogen fixation in this organism [Horner and Burk, 1934].

Horner and Burk [1934] have made a detailed study of some of the other minerals necessary for growth and nitrogen fixation in *Azotobacter*. Their results are given in Table II.

TABLE II

Mineral	Mineral requirements of the organism (in m. mol)	
	In free nitrogen	In combined nitrogen
Calcium	$2-5 \times 10^{-2}$	Negligible
Magnesium	$2-6 \times 10^{-2}$	$2-6 \times 10^{-2}$
Iron	$1.11.6 \times 10^{-2}$	$1.1-1.6 \times 10^{-2}$

It is clear from the results that the requirement of calcium is quite characteristic of nitrogen fixation in *Azotobacter*, since the need for this element is negligible when the organism is not fixing nitrogen; it has also been shown that calcium can be replaced by strontium [Burk and Lineweaver, 1933; Burk, 1932; Horner and Burk, 1934]. It would appear from the above that the element calcium or strontium has a definite role in the mechanism of nitrogen fixation by *Azotobacter*.

Iron has a favourable influence on the fixation of nitrogen by this organism [Kaserer, 1911; Sohngen, 1913; Remy and Rosing, 1911; Bloembergen, 1931]. But the more recent evidence on this point would show that apart from the stimulatory effect that iron exerts on respiration, it plays no special role in the mechanism of nitrogen fixation [Burk, 1934].

In addition to these, a number of other elements have also been studied in this connection. Among these mention may be made of the uranium compounds [Kayser, 1921, 2; Kayser and Delaval, 1924, 1925; Stoklasa *et al.*, 1928]; oxide, nitrate, acetate and the naturally occurring uranium mineral from Belgian Congo are found to exert a marked influence on nitrogen fixation by this organism; thus a concentration of 1 in 50,000 of uranium

The medium increases nitrogen fixation 30-100 fold. Further work is necessary to explain the marked accelerating effect of the minerals, especially compounds of manganese and uranium on nitrogen fixation.

(ii) *Respiration*.—The respiration of this organism is dependent on the presence of sugar in the medium, being 10-15 fold more in its presence than in its absence. Stoklasa [1906] was the first to observe that *Azotobacter* breathes at an enormously high rate. The relation between oxygen pressure and oxygen uptake is unique in *Azotobacter*; thus the rate of respiration is maximum at 0.15 atmos. and diminishes on each side of this rate being 1/3 at 0.005 atmos. and also at 1.0 atmos., the decrease between 0.15 and 1.0 atmos. being linear. The changes are immediate and reversible. This is in striking contrast to most of the cells and tissues hitherto studied; the respiration of yeast for example being independent of oxygen pressure between 0.03 and 0.97 atmos. The respiration per unit dry weight of the organism has a value QO_2 2000 [Meyerhoff and Burk, 1928], whereas it is 1/25 of this value in other forms of bacteria. This rate subsides markedly with increasing age of the culture medium. Narcotics and HCN act similarly on the respiration rate as with other cells. From calorimetric studies Fife [1931] has shown that the rate of respiration remains constant over long periods and that during nitrogen fixation the O/N ratio effecting maximum respiration is the same as when the organism grows in a nitrate medium. The results are not in full accord with those of Meyerhoff and Burk, further confirmation is necessary before any definite conclusions can be drawn from these results.

Burk *et al.* [1932, 2] have shown that respiration is a reversible function of pH (optimum at 7.2 and limits at 5 and 9) and temperature (optimum at 30 and limits at 10 and 50). The temperature characteristic of respiration in *Azotobacter vinelandii* is 19.330 ± 0.115 . Lack of carbohydrate or oxygen causes little permanent injury to respiratory enzyme. Catalase activity is optimum at neutrality but is insensible to acidity or elevated temperature.

Spectroscopic examination of *Azotobacter* cells reveals a respiration mechanism similar to that ascribed to aerobic cells. Keilin [1933] has shown that the Fe compound is the O transporting enzyme and the Fe^{++} and Fe^{+++} are the cytochrome components. With oxidation there is a shift of the band from 632 to 647. A band at 630 which fades on shaking with O_2 and appears on reduction is due to Fe^{++} component in the pigment.

Negelin *et al.* [1934] have studied the shift of bands in relation to narcotics. In spite of its high respiratory quotient, the O transporting enzyme is not affected by carbon monoxide [Keilin, 1933]. The respiration rate is sensitive to HCN and Cu; with the former the effect is reversible, but with the latter the Cu ion forms a solid compound with the cell substance. The effect of HCN on respiration is more marked when the culture is placed for a short time under O_2 deficiency than those having plenty of O_2 .

The respiratory enzyme band being in the red region at 632 instead of yellow, is unique in *Azotobacter*. Such bands are never found to occur in the pheohemins, but occur in the group of compounds which result when the central magnesium of chlorophyll is replaced by Fe. In view of the close similarity of the respiratory enzyme band with that of chlorophyll, it is probable that this enzyme plays

an important part in the fixation of nitrogen in the same manner as the chlorophyll does in the fixation of carbon during photosynthesis. Further work on these lines would lead to results of great value in understanding the mechanism of nitrogen fixation.

(iii) *Environmental conditions*.—The activity of *Azotobacter* and its efficiency for nitrogen fixation are also dependent on the reaction of the medium, temperature and air supply.

In general a reaction below pH 6.0 and above pH 9.1-9.6 is not favourable to the growth of *Azotobacter* [Wenzl, 1934, 2; Martin and Brown, 1938]. The optimum for growth is very near the optimum for fixation [Gainey and Batchelor, 1922; Gainey, 1923] and is different for the different species [Endres, 1934; Willis, 1933, 2].

TABLE III

Species	Optimum pH	Limiting pH
<i>Chroococcum</i>	7.45-7.60	5.8
<i>Beijerinckii</i>	6.65-6.75	5.8
<i>Vinelandii</i>	7.50-7.70	5.9

Thus the activity of the organism in the soil is inhibited by acid secretions from the roots of plants such as maize and wheat [Shelonmova and Menkina, 1935]. Starkey and De [1939] have isolated a new species of *Azotobacter* from soils of India which are acid in reaction (pH 4.9-5.2). Alston [1936] has also reported the presence of a unique strain of *Azotobacter* in Malayan soils, which can tolerate pH 3.6.

There is a marked influence on the amount of nitrogen fixed per gm. mannitol oxidized when the cultures of *Azotobacter* are exposed to light of different colours; in general yellow light is better than blue [Kayser, 1921, 1925; Itano and Matsuura, 1935]. The fact that the organism fixes nitrogen even in darkness shows that the light does not play any fundamental role in the fixation of nitrogen; nevertheless it contributes in some way towards accelerating the process. It would be of interest to find out the mechanism by which light of different wave-lengths thus stimulates nitrogen fixation. It has been claimed by Stoklasa *et al.* [1928] and Kayser and Delaval [1925] that exposure to radio-activity increases nitrogen fixation by *Azotobacter*; in the former case by passing a current of activated air through the culture and in the latter by addition of a powdered radio-active mineral. Stoklasa and Kricka [1928] have shown that β and γ rays depressed the transformation of nitrogen into nucleo-proteins and albumins in the cells of *Azotobacter*, while radium emanation has the opposite effect.

The organism grows and fixes nitrogen between temperatures 10°C. and 50°C., the optimum being 34-35°C. This value is dependent on pH and O_2 tension [Lineweaver, 1933]. Omeliansky [1915, 1926] has shown that

organism can withstand long periods of drought. It has been recorded that nitrogen fixation by *Azotobacter* in tropics takes place at 35°C. [Dhar and Tandon, 1936] and in the Arizona soils at 32·5°C. [Greene, 1932].

Aeration of the medium facilitates nitrogen fixation by *Azotobacter* [Abby, 1907; Hunter, 1922]; thus in a sand medium the organism fixes more nitrogen than in liquid medium [Krainskii, 1910, 1912]. This may be due to quicker oxidation of the sugar, which from the point of view of efficiency may not be necessary.

The presence of other organisms in the medium in which *Azotobacter* grows has been found to exert a stimulating influence on nitrogen fixation by this organism. Thus, in the presence of (a) bacteria, *granulobacter*, *aerobacter*, *radiobacter* and *psuedomonas radiculicola* [Beijerinck and Von Delden, 1902; Bottomley, 1910], (b) protozoa, *amoeba* [Kovats, 1928; Vinogradova, 1928] and (c) algae such as, *oscillaria*, *gleoscapsa* and *chlorella* [Tenechikovosky, 1933], *Azotobacter* fixes more nitrogen. Three times as much nitrogen is fixed in presence of *aerobacter aerogenes* [Kalantarian and Massian, 1930] and in presence of *pseudomonas radiculicola* there is two-fold increase in the amount of nitrogen fixed per gm. of sugar consumed [Bottomley, 1910]. Hanzawa [1914] has found that mixed cultures of different species of *Azotobacter* possess a stronger nitrogen-fixing power than the individual strains.

(iv) *Influence of combined nitrogen on nitrogen fixation.*—The study of the fixation of nitrogen in media containing combined nitrogen has shown that fixation of elementary nitrogen is resorted to by the organism only in the absence of sufficient amounts of available combined nitrogen in the medium [Zoond, 1926; Fuller and Rettiger, 1931], the equilibrium concentration of rapidly available combined nitrogen required to inhibit nitrogen fixation is 0·5 mg. per 100 c. c. [Burk and Lineweaver, 1930]. From a study of the nitrogen changes produced by *Azotobacter* in media containing different nitrogen compounds, Thompson [1934] has shown that they are first converted to ammonia before they are utilized by the organism.

By repeated culturing of *Azotobacter* in a medium containing sodium azoate it has been possible to evolve a strain of the organism which can fix nitrogen even in the presence of combined nitrogen in the medium [Remzer, 1938]. The nitrogen-fixing capacity of the organism is suppressed by growing it for long periods in media containing potassium nitrate [Stumbo and Viney, 1938]. In this connection it would be of great interest to study the change in the azotase system under the above methods of culturing the organism.

(v) *Products of metabolism.*—In dextrose media *Azotobacter* produces lactic, acetic, lactic and tartaric acids and ethyl alcohol, a large part of it being converted into carbon dioxide [Ranganathan and Norris, 1927; Aso *et al.*, 1932]. Phthalic acid has also been detected in *Azotobacter* cultures [Aso, *et al.*, 1932]. Siffred and Anderson [1936] have found that a mixture of sterols allied to ergosterols and the water-soluble vitamin B₁ are elaborated by this organism in culture medium. As compared with other types of soil bacteria there is nothing unique in the products of metabolism of this organism.

The various physiological functions (respiration, growth and efficiency) being quite similar when the organism grows in free or combined nitrogen, no conclusion can be drawn from these on the chemical mechanism of nitrogen fixation.

Chemical analysis of Azotobacter cells

In general the composition of the cells does not vary greatly from that of other common bacteria. The cells consist chiefly of carbohydrates, proteins and a small percentage of minerals. The proportion of these various constituents vary with the different species as also with the physical conditions of the medium in which the organism is grown [Hoffman, 1913; Omeliansky and Seiber, 1913].

(i) *Nature of proteins*.—A typical analysis of the cells with special reference to the proteins [Greene, 1935] is given in Table IV.

TABLE IV

Species	As percentage on dry cell material			As percentage on total nitrogen			
	Carbo- hydrate	Protein	Ash	Amide N	Humin N	Basic N	Filtrate N
<i>A. chroococcum</i>	62.34	25.00	4.00	17.90	9.62	20.00	52.5
<i>A. beijerinckii</i>	64.72	24.68	4.05	18.25	13.67	25.94	42.0
<i>A. agile</i>	22.09	61.19	7.55	13.42	13.18	26.90	45.7
<i>A. vinelandii</i>	25.67	52.25	7.66	20.36	15.82	22.22	41.8

The Van Slyke analysis of the proteins have shown that in general the cells contain a large amount of arginine varying from 16 to 20 per cent.

It is evident from the table that there is a close similarity between *chroococcum* and *beijerinckii* on the one hand and *agile* and *vinelandii* on the other with regard to their general chemical composition. Although more of carbohydrate is synthesized by the first two, they fix comparatively less amount of nitrogen than *agile* and *vinelandii*. It would appear from the above that carbohydrate elaboration in the cells of these organisms has no significant effect on nitrogen fixation.

Hoffman and Hammer [1910] have found that the amount of nitrogen in dry cell material increased as the incubation period advanced, being 1.0 per cent in seven days and 2.84 in 21 days. This shows a very high content of non-protein material which though not in agreement with Stoklasa was confirmed later by Omeliansky and Seiber [1913].

It has been found that the proteins of *Azotobacter* form a better source of nitrogen for alcoholic fermentation [Kayser, 1921, 1]. The close similarity in the amino acid make-up of *Azotobacter* proteins and those of leguminous plants [Greaves and Reeder, 1935] would suggest that there is some parallelism in the fixation and assimilation of nitrogen in these two systems. A comparative study of the proteins formed by this organism when grown

media containing free and combined nitrogen has not so far been attempted. Study of the change in the complexity factor of the proteins of *Azotobacter* at various stages of growth and nitrogen fixation in these media would throw light on the chemical steps through which the protein is synthesized in this organism.

(ii) *Slime production*.—When grown in culture media this organism produces a characteristic slime; the slime is essentially a carbohydrate, levorotatory, resistant to hydrolysis by acids, and belongs to the class of true gums (Inborn and Hamilton, 1929). The production of gum varies in the different species and is increased by having more complex carbohydrates in the medium (Hamilton, 1931).

The role of slime in the mechanism of nitrogen fixation is not very clear. Maskaran [1936] has shown that the cells and slime of *Azotobacter* exhibit definite and unique C/N relationships during the growth of this organism in culture media. The greater intake of carbon by the cells and slime production during the early stages of growth would suggest that the formation of slime is a necessary precursor for nitrogen fixation which follows later on. Study of the change in C/N of the bacterial cells freed from slime would throw further light on this point.

Burk [1934] has observed that high nitrogen pressure is necessary for the enzyme catalysis resulting in the fixation of nitrogen; thus a concentration of 1.6×10^{-4} M of nitrogen is necessary in the medium for successful enzyme action. The solubility of nitrogen gas in water being very low, it is probable that the slime may serve as an efficient medium for adsorbing the nitrogen gas before it is fixed. In view of this, it would be useful to study the solubility of nitrogen gas in the slime. The production of slime when the organism grows in a medium containing combined nitrogen (when the organism fixes no N) is also a point of great interest.

(iii) *Pigment formation*.—With ageing in the culture medium the different species of *Azotobacter* form characteristic pigments; *A. chroococcum* forms a brown pigment, *A. agile* a fluorescent dye and *A. vinelandii* a yellow, water-soluble greenish fluorescent pigment similar to the flavone pigments (Betschenko, 1930; Ellinger and Koschura, 1933). Pigment formation is greatly affected by the nature of the nutrients present in the culture media: the presence of dextrin, chalk, $\text{Ca}(\text{NO}_3)_2$ and increased air supply facilitate pigment formation; and traces of MnSO_4 , ZnSO_4 and exposure to ultra-violet rays reduce early pigmentation and shorten life in the organism [Omeliansk and Terova, 1911; Hills, 1918; Colley, 1931; Itano and Matsuura, 1935]. In the case of *A. vinelandii*, pigment formation takes place only in the presence of glycerine in the medium. The presence of small amounts of acetone in the medium of *A. chroococcum* and sodium acetate in the case of *A. agile* inhibits pigment formation [Wenzl, 1934, 1].

The pigment is a melanin of unknown nature formed from tyrosine by the action of tyrosinase [Umgerer, 1934]. The significance of this characteristic pigment formation in the life of the organism is not very clear. Probably with advancing age and death of the organism, conditions in the cell become more favourable for the action of the tyrosinase present in the organism to convert tyrosine into melanin, thereby conserving the nitrogen in a more resistant form in nature.

Biochemical mechanism of nitrogen fixation in Azotobacter

Our knowledge of the biochemical mechanism by which the organism fixes nitrogen is still meagre. The work done so far in this direction can be conveniently dealt with under the following heads :—

(i) *First demonstrable chemical step in nitrogen fixation.*—Various nitrogenous compounds have been detected in *Azotobacter* cultures fixing nitrogen and these have been supposed to be the first intermediate product in nitrogen fixation.

Gautier and Drouin [1888] and Bonema [1903] were first to suggest that fixation takes place by direct oxidation of the free nitrogen to nitrate. A few workers have also detected nitrate in cultures; but it has been shown that the phenoldisulphonic acid test for nitrates which these workers have adopted is not reliable because of the presence of pigments [Kellerman and Smith, 1912]. A number of workers have also subsequently failed to detect the presence of nitrates or nitrites in the medium in which fixation took place. Direct experimental proof in support of oxidation of nitrogen to give oxides, NO_2 and NO_3 , is still lacking.

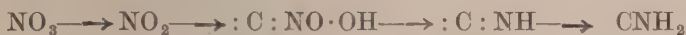
The presence of ammonia has been detected in *Azotobacter* cultures by a number of workers and this is generally considered to be the first demonstrable stage in nitrogen fixation [Kostychev and Ryskaltchout, 1925; Ranganathan and Norris, 1927; Halverson, 1927]. Wieland [1922] considered that the action of hydrogen accepters formed in the cells of nitrogen-fixing bacteria does not depend upon oxygen for hydration but upon molecular nitrogen with which it forms ammonia perhaps through the hydrazine stage in a manner similar to Haber process. Winogradsky [1930, 1, 2] has demonstrated the formation of ammonia by an ingenious culture technique, using alkali salts of organic acids as nutrient for the organism. Kostychev *et al.* [1926] have adduced evidence to show that the fixation in *Azotobacter* is an extra-cellular reduction process and Winogradsky [1932] has further suggested that the ammonia is formed by the action of an enzyme—a dehydrogenase—with the aid of a catalyser, perhaps by symbiotic action. Kostichev *et al.* [1926] and other workers have confirmed these findings. By mixed culture studies of *Azotobacter* and other ammonia utilizing organisms, like mycoides, in nitrogen-free media, Novogradskii [1933] has adduced additional evidence for the formation of small quantities of ammonia in the medium.

This question of ammonia formation has attracted much attention in recent years. Burk and collaborators [1935, 2; 1936, 1] have critically examined the origin of ammonia detected by these workers and have come to the conclusion that this is due to the secondary oxidative de-amination of the bacterial protein which takes place in the medium side by side with nitrogen fixation. Isakova [1933] has shown that the amount of ammonia formed in the presence of glucose remains fairly constant with negligible variation, but increases after glucose disappearance and that this ammonia production is more marked in acid cultures due to autolysis of cells. But, on the other hand, a number of workers have reported that this secondary ammonia formation does not take place in presence of sugar in the medium. In view of the above conflicting evidence it would be difficult to draw any definite conclusion in regard to the origin of ammonia in the cultures.

By means of *in vitro* experiments using Pt and Pd as catalysts Knoop [1927] has suggested that where ketonic acids and ammonia meet in the cell under conditions favourable for hydrogenation, amino acids are formed very easily.

Hydroxylamine, hydrazine and amide have been reported from time to time as the first intermediate stage in nitrogen fixation, and among these the hydroxylamine theory needs some consideration. Blom [1931] from theoretical considerations has suggested that hydroxylamine is first formed with Fe catalyst. In the light of this theory he has explained the checking action of NO_3 and NH_3 on nitrogen fixation. The conclusion arrived at by Burk regarding the non-specificity of Fe in fixation would suggest that Blom's idea of iron catalysts requires revision.

Recently Endres [1934, 1, 2] has adduced further evidence in support of the hydroxylamine theory. In lactate cultures he has detected 1.2 γ of hydroxylamine. He has shown that this is formed by the hydrolytic decomposition of oximes. In *Azotobacter* cultures fixing nitrogen the oxime concentration increases from 0.5 to 12 ml/litre in 72 hours [Endres, 1934, 3], whereas in the absence of elementary nitrogen there was no increase in the oxime concentration. By studies in nitrate cultures, Endres and Kauffmann [1938] have further suggested a scheme of formation of oximes and amino acids from NH_2OH as follows:



On the other hand, Burk and Horner [1935, 1] have reported that the compound NH_2OH and oximes beyond 3 mg./litre are toxic to the growth of *Azotobacter* and that NH_2OH is not produced in nitrate cultures.

Direct union of nitrogen with carbon compounds to form amino acids has also received some attention in recent times. A number of workers (Waynick and Woodhouse, 1922; Halverson, 1927; Kumagawa, 1928) have detected amino acids in *Azotobacter* culture media. Among these the observations of Virtanen and Laine [1938] are interesting. By analogy from gumme fixation and as a result of direct experiments with *Azotobacter*, he has suggested that asparatic acid is formed before ammonia in the medium, the asparatic acid being formed through the oxime of oxal-acetic acid. Direct experimental evidence of its formation has been adduced by analysis of copper salt of the oxime and oxyacids have also been detected in cultures. The asparatic acid thus formed later on yields ammonia. This theory agrees with the oxime theory of Endres and also accounts for the ammonia formed in culture media. From the evidence so far available it would appear that this is the most probable mechanism by which the N_2 molecule is fixed by the free-living bacteria, nevertheless conclusive evidence on this point is still wanting.

(ii) *Enzyme systems*.—Bakh [1934] made a sensational announcement that the liquids containing the enzyme obtained by filtering *Azotobacter* cultures at 300 atmospheres through Chamberland L_3 candles fixes atmospheric nitrogen in presence of sugars. His results are presented in Table V.

The results show fixation of nitrogen in cell-free cultures. This observation has not been further confirmed. The present authors have obtained

evidence (unpublished data) to show that cells treated with antiseptics chloroform and toluene, fix considerable amounts of nitrogen in sugar media

TABLE V
Amount of nitrogen fixed by Azotobacter cultures

Time in days	Nitrogen in mg.		
	Control	Glucose	Mannitol
0	1.976
9	2.783	14.683	10.645

Certain amount of definite information is available regarding the properties and behaviour of the enzyme system fixing nitrogen in *Azotobacter* cells, as a result of the researches of Burk and co-workers. They have studied the enzyme system by means of the well-known Warburg manometric technique using living cultures of the organism. The studies have been made with special reference to the auxillary substances and environmental conditions necessary for the enzyme action. The properties of the enzyme have been defined on the basis that the metabolic activities of the organisms obtaining their nitrogen from nitrogen gas are specifically ascribed to the fixation process and that the organisms obtaining nitrogen from combined nitrogen do not behave similarly.

Burk and co-workers [1934, 1, 2, 3] have considered the enzyme system fixing nitrogen in *Azotobacter* as a phyto-enzyme (an enzyme whose activity is correlated with the structure of the living cell to the extent that the velocity of formation of the intra-cellular products parallels and is normally measured by the velocity of growth) and has named it as azotase. The specific component within the azotase system which combines with the N_2 molecule is termed nitrogenase, (E). The fixation of nitrogen at ordinary temperatures and pressures by *Azotobacter*, as a function of the nitrogen pressure, corresponds to one N_2 molecule combining reversibly with one enzyme molecule E (nitrogenase) to form a compound N_2E which later reacts to yield protein according to equation, $N_2 + E \longrightarrow N_2E \longrightarrow P$ [Lineweaver *et al.*, 1934]. The thermodynamic dissociation constant of the above reaction, KN_2 ($[E][N_2]/[N_2E]$) has been found to be 0.215 ± 0.002 atmospheres. This corresponds to 1.64×10^{-4} M of dissolved nitrogen gas in the medium at $31^\circ C$. Compared to most other enzyme reactions in which gases are involved, such as photosynthesis and respiration (where kCO_2 and kO_2 are of the order of 10^{-5} atmospheres), a large nitrogen pressure appears to be needed for enzyme reaction.

From energy considerations and entropy they have shown that the decomposition of N_2E is a bimolecular reaction and only a negligible fraction of the total amount of carbohydrate is used for nitrogen fixation by the enzyme system under thermodynamically ideal conditions, thereby showing the

ably carbohydrate plays no role in the enzyme mechanism of nitrogen fixation. The relationship of the dissociation constant under varying conditions of enzyme action would point out that more than one active intermediate is not formed in the system. The free energy of dissociation of N_2E has been found to be 100—1,000 calories.

The dissociation constant is quite characteristic of the reaction of the calcium, being independent of the following factors: concentration of calcium, strontium, barium, iron and indifferent narcotics such as alcohols urethanes and urea derivatives; pressure of oxygen; temperature; pH ; and certain physiological factors such as the species of *Azotobacter*, concentration and age of the culture. The concentration of calcium, strontium, oxalate and pH are known to bear a specific relation to the mechanism of nitrogen fixation as distinguished from growth. Nitrogen fixation by azotase decreases from a maximum at pH 7.8 to a zero limit at 5.97 ± 0.02 , and irreversible inactivation occurs at pH below 5.0. The rate of decomposition of fixed nitrogen decreases from a maximum at pH 7.8 to a limit at pH 4.5. The rate of O_2 consumption as function of pH is similar in type. The pH value for fixation is a characteristic constant independent of other reactions. Results on the study of phase changes with special reference to azotase activity in relation to pH would suggest that the nitrogen-fixing system is a two-component heterogeneous system of three phases in equilibrium at a critical pH .

They have also adduced evidence to show that Ca replaceable by strontium and molybdenum replaceable by vanadium are specific for nitrogen fixation by azotase; Fe does not play any part in the process of nitrogen fixation by azotase.

By studies in free and combined nitrogen they have further shown that the enzyme systems responsible for assimilation of free and fixed nitrogen are different although considerable relationship exists between them. A more specific nitrogen-fixing enzyme E with which N_2 combines before forming N_2E is also postulated.

From a study of the influence of various nitrogen pressures on nitrogen fixation by *Azotobacter*, Burk [1930] has found that the effect is immediate and reversible, being proportional to N_2 pressure. The effect is felt to an appreciable extent at 0.5 atmosphere with limits at 5—10 atmospheres.

Nitrogen fixation by azotase is also proportional to O_2 tension in the medium; the efficiency ratio (nitrogen fixed/ O_2 consumed) increases 10 to 100 fold between 0.21 and 0.01 atm. O_2 and the rate of nitrogen fixation attains a maximum at 0.04 atm. O_2 being only $\frac{1}{3}$ or $\frac{1}{6}$ at 0.008 and at 0.21 atm. The influence of O_2 is not affected by the presence of other gases such as H_2 and N_2 . The nitrogen obtained from *Azotobacter* suspensions by vacuum fractionation at low temperatures obeys typical Henry's law relationships and is similar to those obtaining in legume bacteria and yeasts.

A certain amount of work has also been done on the other common enzymes present in *Azotobacter*. The cells are found to be rich in catalase [Burk *et al.*, 1932, 2], numerous dehydrogenases [Lineweaver *et al.*, 1932], xanthine oxidase [Meyerhoff and Schulze, 1932; Keilin, 1933; Negelein and Gerisher, 1933], the spectroscopically observable 'atmungs ferment' [Negelein and Gerisher, 1933], malonate carboxylase and the newly observed

co-enzyme R [Allison, 1933]. Nilsson [1936] has found that in mannitol *Azotobacter* develops a dehydrogenase which is absent when the organism grows in glucose; hexose phosphate dehydrogenase is present in both cases.

The extra-cellular isolation of azotase and the study of the enzyme apart from the growth and general metabolism of the organism have not so far been possible and any advance in this direction would greatly help to understand the enzyme mechanism responsible for nitrogen fixation.

Nitrogen fixation in clostridium

(i) *Distribution of clostridium*.—Winogradsky [1893] isolated a sporogenous anaerobic organism, known as *Clostridium*, from the soil of St. Petersburg which has the power of assimilating nitrogen and bringing about the butyric fermentation of carbohydrates. He further demonstrated the widespread occurrence of this organism in the soils of St. Petersburg and Paris. Omeliansky demonstrated the presence of this organism in almost all Russian soils that he examined. This was also present in most of the German soils examined [Freudenreich, 1903]. Haselhoff and Bredmann [1906] have found that in nearly all samples of soils and leaf moulds examined, *Clostridia* were present. They are found in rather large numbers in acid soils. Addition of CaCO_3 or CaO to the soil had little effect on the numbers of *Clostridia* or the amount of nitrogen fixed. It is much more abundant than *Azotobacter*, the number being over 100,000 per gm. of soil [Truffaut and Bezssonov, 1921].

Clostridium is as important as *Azotobacter* in nitrogen fixation [Omeliansky, 1906]. Truffaut and Bezssonov [1921] have suggested that it is *Clostridium* and not *Azotobacter* that is the principal agent for the fixation of nitrogen in the soil. In the acid soils of Iowa, the anaerobic nitrogen-fixing organisms are of considerable importance in maintaining the fertility; it can be found in soils even with a pH of 5.0 — 5.2. It is of interest to note in this connection that although considerable amounts of nitrogen are fixed, there is little increase in ammonia or amino nitrogen [Walker and Willis, 1933]. The number of *Clostridia* present in the soil can be increased considerably by partial sterilization using CaS and heat.

(ii) *Morphology*.—Bergey [1930] defines them as: 'Anaerobes or microaerophiles, often parasitic. Rods frequently enlarged at sporulation producing *Clostridia* or *Pleocidia* forms'. A few of these have the power of fixing nitrogen and *Cl. pasteurianum* is typical of the species fixing nitrogen.

Winogradsky found *Cl. pasteurianum* to be an obligate anaerobic form which can develop under aerobic conditions only in presence of aerobic bacteria. However, the facultative aerobic *Cl. americanum* isolated by Pringsheim [1906] was very similar in morphology to the *Clostridia*, but was capable of fixing large quantities of nitrogen, perhaps due to its aerobic nature.

Bredmann [1912] after examining a large number of species of *Clostridia* came to the conclusion that the various strains classified into different groups belong to one and the same species, viz., *Bacillus amylobacter*, the difference being due to the methods of isolation and treatment. It is easy to bring about a regeneration of the nitrogen-fixing capacity. The latter observation has, however, been questioned by Pringsheim.

McCoy *et al.* [1928] have studied this group of organisms and have recognized three sub-groups—*Cl. pasteurianum*, *Cl. saccharo-butyrus* and the *Clostridium* type. They have found that the different groups fix varying amounts of nitrogen in culture media; *pasteurianum* fixes 0.66 - 3.98, *glycicum* 0.64 — 2.35 and *plectridium* 0.65 — 2.78. The differentiation of these is not well defined.

(iii) *Cultivation and cultural characteristics.*—A modification of Winogradsky's nutrient medium adding soil extract, small amounts of $MgSO_4$ and H_2HPO_4 along with traces of Fe and Mn salts and sufficient $CaCO_3$ has been found useful for the cultivation of *Clostridium*. It can be easily isolated after prolonged pasteurization at 75°C.

Itano and Arakawa [1930] have recommended a method for the culture of *Cl. pasteurianum*; the method is but a modification of the Morse-Kopeloff culture of anaerobes. Partial sterilization leads to increased nitrogen fixing power in *Clostridia*, probably due to the destruction of substances harmful to them [Truffaut and Bezssonov, 1921]. The optimum temperature for the development of *Cl. pasteurianum* is 28° — 30°C. The optimum reaction is pH 6.9 — 7.3, but the organism still develops well at pH 5.7. It has been observed that it can withstand a greater acidity than proteolytic anaerobes like *Bacterium putrificus*, from which it can thus be freed. Addition of $CaCO_3$ to the glucose medium has a favourable effect in neutralizing acids formed and doubling the amount of nitrogen fixed [Truffaut and Bezssonov, 1922]. In this connection $MgCO_3$ is found to be less favourable. At pH 5 — 9.5 *Clostridium* fixed 4 — 4.3 mg. per 50 c. c. medium in three weeks; at pH 5.0, 3.2 mg. of nitrogen was fixed. $CaCl_2$ has also been found to be useful in promoting nitrogen fixation [Willis, 1933, 1]. Willis [1933, 2], however, has shown that pH of the medium has little effect on the amount of nitrogen fixed in the medium between pH 5.0 — 9.5. Omeliansky [1906] has found that the organisms are very active at 30°C. and fix less nitrogen at higher temperatures.

When freshly isolated, the *Clostridium* fixes more nitrogen than when cultivated for a long time in artificial media. The culture can be invigorated by growing it in Winogradsky's medium to which enough ammonium sulphate is added so as to offer the organism less nitrogen than is needed for the complete decomposition of the sugar. By transferring from this culture, when gas formation ceases, normal growth and nitrogen fixation is obtained [Bredmann, 1909]. Bredmann has further shown that the culture could be invigorated by passing it through soil.

Spores are formed when the organism is grown in medium with plenty of oxygen; the presence of 30 mg. of oxygen per litre of air will still allow spore formation. The spores are not destroyed at 75° even at the end of 5 hours; at 100° the spores are destroyed in five minutes. The spores could be preserved in the dry state for 20 years with the nitrogen-fixing power intact [Omeliansky, 1906].

(iv) *Fermentation of carbohydrates.*—The fermentation of various carbon compounds depends on the nature of the nitrogenous nourishment. Dextrose, sucrose, levulose, inulin, galactose and dextrin are fermented in presence of ammonium sulphate, while only dextrose, sucrose and inulin are attacked when ammonium sulphate is present. Lactose, arabinose, starch, gum, mannitol, dulcitol,

glycerol and calcium lactate are not attacked by the organism under any conditions [Winogradsky, 1902]. *Clostridium* does not attack cellulose while in presence of cellulose-destroying bacteria more nitrogen is fixed than with carbohydrate alone [Pringsheim, 1913]. Omeliansky [1906] has observed that glycerol, mannitol, maltose and raffinose are also fermented by this organism. Peterson *et al.* [1926] have studied the fermentative powers of *Cl. thermocellum* on nine sugars and five related compounds. Stickland [1934] has carried out experiments on the chemical reactions by which *Cl. sporogenes* obtains its energy.

The characteristics of the butyric fermentations brought about by the organism are such that 42-45 per cent of the dextrose is converted into a mixture of acetic and butyric acids in varying proportions; small amounts of alcohol, ethyl, propyl and isobutyl, are formed, and a considerable evolution of a mixture of CO_2 and H_2 occurs [Winogradsky, 1902]. Acetyl-methyl carbinol is formed by *Clostridium acetobutyricum* along with acetic and butyric acids. Its production can be increased by the addition of phosphate and decreased by proteins and is more closely associated with the formation of acids than that of acetone and butyl alcohol. Wilson *et al.* [1927] have shown that acetyl-methyl carbinol is a regular end product of the fermentation, it being formed at the same time as acetic and butyric acids and all three probably have the same precursor. With fermentation the acidity of the medium changes to approximately *pH* 3-4. Butyric acid forms the larger part of the volatile fraction of the acids [Walker and Willis, 1933]. The amount of acid produced is correlated with the amount of glucose utilized. It produces large amounts of CO_2 under anaerobic conditions in a medium containing CaCO_3 . The main source of carbon dioxide is the glucose molecule though small amounts result from CaCO_3 [Willis, 1933, 1].

(v) *Anaerobic nitrogen fixation*.—In nitrogen-free medium *Clostridium* fixes about 3 mg. of nitrogen per gram of sugar decomposed [Winogradsky, 1902]. Haselhoff and Bredmann [1906] have found that both crude and pure cultures of anaerobic bacteria isolated from soil absorb quite considerable amounts of nitrogen similar to that reported by Winogradsky. For each gram of sugar fermented 3-6 mg. of nitrogen was assimilated. Too large an increase in the nitrogen content of the medium decreases nitrogen fixation and finally stops it entirely, but nitrogen fixation still takes place when the ratio of N : sugar is 16 : 100, whereas according to Winogradsky this ratio is 6 : 1000. *Cl. acetobutylicum* (Weizman) fixes comparatively little nitrogen; the four strains studied fixed from 0.69 to 1.06 mg. in 100 c. c. medium. From a study of single cell culture of *Clostridium*, McCoy *et al.* [1928] have correlated nitrogen assimilation with consumption of sugar although the relative efficiency varies with the stage of growth. The greater the concentration of sugar the lower is its economic utilization, 3.2 mg. of nitrogen being fixed per gram of glucose in 0.5 per cent solution, 2 mg. in 2 per cent solution and 1.2 mg. in 4 per cent solution [Waksman, 1931].

Combined nitrogen in the medium reduces nitrogen fixation, NaN_3 having the least effect. No nitrogen is fixed in the presence of ammonium sulphate and in peptone only 0.6 mg. of nitrogen is fixed in 25 days. The production of CO_2 is directly associated with the sugar utilization, the amount

ing the same whether the organisms are grown in an atmosphere of nitrogen or air [Walker and Willis, 1933; Willis, 1933.2]. Glucose was rapidly used in media containing peptone, ammonium sulphate or NaNO_3 , but little or no nitrogen was fixed. CaCO_3 may act as a neutralizer or an acceptor for H in anaerobic fermentations [Willis, 1933.1]. In media containing 0.2 CaCl_2 or 2 gm. of CaCO_3 per litre comparatively large amounts of nitrogen are fixed in solution. NO_2 and NO_3 are formed in presence of CaCO_3 but not in culture media containing CaCl_2 [Willis, 1933.2]. When the solution contains more than six parts of combined nitrogen in one thousand parts of solution, nitrogen fixation comes to a stand still. Omeliansky has, however, obtained nitrogen fixation even with a concentration of 16 parts of combined nitrogen in 1000 medium.

Comparatively more of carbohydrate is used for nitrogen fixation by the anaerobic bacteria. But if the energy liberated rather than the carbohydrate used is taken into consideration, the anaerobic species are as efficient as the aerobic ones from the point of view of energetics of nitrogen fixation.

Nitrogen fixation in other free-living bacteria

It has been claimed from time to time that a large number of organisms in the soil, other than *Azotobacter* and *Clostridium*, have the power of fixing nitrogen [Greaves, 1929, 1930; Emerson, 1917].

Lichtenstein *et al.* [1907] have described a new aerobic nitrogen-fixing *Clostridium* having only about half the capacity of the previously described *Clostridia*. Truffaut and Bezssonov [1925, 1] have isolated a new bacillus from soil which fixes 2-7 mg. of nitrogen per gm. of carbohydrate. *Bacillus suffanti*. Lipman [1938] has isolated some new non-symbiotic bacteria from the white sands of New Mexico, which have the power of fixing nitrogen.

SYMBIOTIC FIXATION OF NITROGEN BY NODULE BACTERIA

Bouissingault [1838] pointed out that leguminous plants absorb atmospheric nitrogen, basing his conclusion on the experiments he conducted with clover and wheat. But not until 1888 it was known by the classical researches of Hellriegel and Wilfarth that these plants could gain atmospheric nitrogen through the nodules present in their roots. They showed that nitrogen is fixed because of the presence of a species of bacteria known as *Bacillus radicola* in the nodules. Following up this important piece of work, Beijerinck succeeded in isolating this organism from the plant, and attempted to study the symbiosis between the bacteria and the host plant. Since then a large volume of work has been done on this bacterium, but our knowledge of symbiotic nitrogen fixation is still meagre.

Ecology of legume bacteria

(i) *Occurrence*.—The legume bacteria, as the name would suggest are generally present in soils in association with plants belonging to the natural order Leguminosae [Edwards and Barlow, 1909]. There are, however, a few plants in this natural order which are not infected by the bacteria [Leonard, 1925]. Occasionally the bacteria are also found in the roots of non-leguminous plants such as the Russian olive tree, *Elanotus americanus*, and *Surina* [Snyder, 1925; Blake, 1932].

(ii) *Nomenclature*.—The nodule bacteria are referred to by different names according to the particular system of classification adopted. Bergey [1930] places all the known nodule bacteria under the genus *Rhizobium* and six species have been recognized in this group, viz., *Rh. leguminosarum* Frank, *Rh. trifolii*, *Rh. phaseoli*, *Rh. meliloti*, *Rh. radicola* and *Rh. japonicum*.

(iii) *Morphology and life-cycle*.—The general characteristics of the above group of organisms have been defined by Bergey [1930] as 'minute rods, motile when young, branching rods abundant and characteristic when grown under suitable conditions, obligate aerobes capable of fixing atmospheric nitrogen when grown in the presence of carbohydrates and in the absence of organic nitrogen compounds, produce nodules in the roots of leguminous plants'.

Whether grown in culture media or soil, the bacteria exhibit a clear and definite life-cycle. According to Bewley and Hutchinson [1920] the life-cycle consists of five stages: (a) the small non-motile pre-swarmer coccus, (b) the larger non-motile coccus, (c) motile swarmer, (d) rod form and (e) the stage of high vacuolation (bacteriod). In the nodule tissue cocci, small evenly staining rods and banded granular rods usually occur at successive age levels in the growing nodule. In the nodule, the granular rod stage consists of swollen pear-shaped and banded cells which are called bacteriods. Striking deviations in the life-cycle have been described by Gibson [1928], but more work is needed before the less common cell types can be established as normal components of the life-history. Gangulee [1926] has reported that in culture media all the various stages are found to occur simultaneously but in varying proportions. The distinct life-cycle has a bearing on the spread of bacteria through the soil and consequently on the infection of the host plant [Thornton and Gangulee, 1926]. It helps in the movement of the organism. The amount is not detectable until the majority of the organisms have passed into the coccoid stage and is thus presumably due to the active migration of flagellated swimmers [Thornton, 1936, 3]. Banded rods are the most common form met with in the life-cycle of the groundnut-nodule organism [Rajagopalan, 1938].

Lewis [1938] has studied the cell inclusions of *Rhizotia* and has concluded that the life-history of the organism is not cyclogenic in the sense that special reproductive cells, gonidia or spores are formed in the process of reproduction.

(iv) *Cultivation and cultural characteristics*.—These organisms can be grown in culture media having the following composition [Wieland, 1922]: Mannitol 10 gm., NaCl 0.2 gm., K_2HPO_4 0.5 gm. $MgSO_4 \cdot 7H_2O$ 0.2 gm. $CaSO_4 \cdot 2H_2O$ 0.1 gm., $CaCO_3$ 1 gm. and yeast water 100 c.c. in a litre of medium. Suitable modifications of this medium have been made by different workers for isolation and cultivation of this organism [Edwards and Barlow, 1909; Carrol, 1934, 2; Whiting, 1915].

Attempts have been made to study the cultural characteristics of the different strains of organisms present in various species of plants by growing them in artificial media. Thus Stevens [1925, 1] has found that the strains of alfalfa and sweet clover studied by him are divisible into two groups based on their cross-agglutination test and nitrogen-fixing power in sand cultures. He [1925, 2] has also observed that litmus milk is more useful than janus green or cresol purple in bringing out the characteristics of these groups. From the physiological reactions Wright [1925] concludes that these do not

represent two distinct species but two biotypes each of which varies around a type of its own. Organisms of the groundnut nodule are also divisible into physiological groups [Rajagopalan, 1938]. Madhok [1935] has studied the cultural characteristics of the organism causing nodules on the roots of alfalfa (Egyptian clover).

When grown in sugar media, organisms of alfalfa, clover, pea and vicia produce an acid reaction, while those of soyabean, cowpea and lupine produce an alkaline reaction [Baldwin and Fred, 1927; Bushnell and Sarles, 1937]. Most of the wild legumes produce an alkaline reaction in sugar media [Conklin, 1936]. Sivasubramanian and Bari [1936] have reported that Indian nodule organism isolated from *Cajanus indicus* produces both alkaline and acid reactions depending on the nature of sugars in the medium.

Classification based on fermentation characteristics of the organisms is in harmony with flagellation, or other cultural and serological reactions [Baldwin and Fred, 1927; Clarke and Hansen, 1933; Carrol, 1934, 1]. Anderson and Carrol [1932] have suggested that viscosity in solution cultures may also be of value in differentiating *Rhizobium* cultures.

The H-ion concentration of the medium exerts considerable influence on morphological characters and growth of the organism, thus *Rh. radicicola* isolated from kidney bean, red clover, cultivated pea and hairy vetch do not grow in media above pH 7.0 or below pH 4.5 [Smezkov, 1938].

Among the serological reactions of this organism, the complement fixation and agglutination test are of equal value in identifying the different strains. It is of interest to note in this connection that a close protein kinship exists among the strains isolated from 15 species of *Crotolaria* [Carrol, 1934].

Burke and Burkey [1925] have found that by growing *Rhizobium radicicola* in increasing concentrations of dye, the organisms are modified to give a rate 1 in 1000 of genitain violet.

West and Wilson [1938, 1, 2] have shown that vitamin B₁, an active heat-labile substance and riboflavin are synthesized by growing cultures of *Rh. radicicola*.

Relationship between leguminous plant and nodule bacteria

(i) *Infection of the host plant*.—Simultaneously with the unfolding of the true leaf, the root secretes a substance, probably of the nature of a mucilage, and at this stage the first appearance of the nodule on the seedling takes place. The nature of the secretion as also the seat of its formation are not known. The removal of the leaf does not delay nodule formation [Thornton, 1929], probably the coincidence is incidental and the unfolding of the leaf may not have any direct bearing on nodule appearance.

Thornton [1936, 1] has worked out the histological changes that take place in the root tissue during infection. The nodule bacteria secrete a thermostable, water-soluble active substance which comes in contact with the root tip and produces a characteristic curling [Thornton and Nicol, 1936]. This curling of the root tip is a necessary prelude to infection [McCoy, 1932]. In the early stages a small colony of bacteria appears close to the apex of the root. This causes irregular growth of the root hair, so that the hair curls over to form a tight spiral. As a result of this, a local weakening of the cell-wall takes place

and the bacteria enter the root at this point. In liquid cultures *Rhizobium* induces hyperplastic effect on the roots of peas; it also causes hypertrophy with the deformity of cortical cells and is dependent on the elongation of cells [Mollard, 1913].

There is a marked specificity in the infection of host plant by bacteria. Eighteen host specific groups have so far been recognized. Infection of host plant outside the host specific groups have been described [Allen and Allen, 1939], but it is of very rare occurrence. The serological reactions of nodule organism have been correlated with host specificity [Baldwin and Hiltner, 1927] and ability for cross inoculation also corresponds with the serological and other biochemical reactions of the bacteria [Walker, 1928]. There is a correlation between the amount of indol-acetic acid produced in synthetic media in presence of tryptophane by various strains of *Rhizobium* and ability to induce formation of nodules on the roots of leguminous plants [Georgi and Bond, 1939]. The immunity is not connected with the preliminary curling of the root hair—probably it is a protein reaction.

(ii) *Nodule formation*.—When the bacteria have penetrated into the root hair they form a thread-like growth of zoogloea, known as the infection thread, which passes down the hair and penetrates the cortex of the root. This causes the root cortex to become meristematic and by division to produce the young nodules. The cell division may extend inwards to involve the endodermis and even the pericycle cells. This might have an important bearing on the diffusion of nutrients into the nodules. Cell division extends beyond the zone that are actually infected and is perhaps due to the secretion of some stimulus by the bacteria.

The bacteria are distributed through the cells of the young nodules in three different ways in different legumes [Milovidov, 1928]. In the first type, common to most legumes, the bacteria spread by means of infection threads which pass through the perforations in the cell wall. Thornton [1936] has observed that secondary release of bacteria may take place in this type of distribution by the formation and subsequent breaking up of blisters formed from the infection thread. In the second type which occurs in *Serratula*, the bacteria infect the intercellular spaces and spread by that means. The third type is met with in lupins, in which the bacteria invade the dividing cells in the young nodules and are thus distributed in the daughter cells.

Ultimately the presence of bacteria in the host cells stops their division while permitting them to increase in size. The combined activity of uninfecting cells causes further growth of nodules, and this seems to be essential for the healthy functioning of the nodule. The presence of the nodule in the cortex induces an outgrowth of vascular strands from the stele. A second endodermis is formed surrounding each vascular strand and enclosing the central infected tissue as far distally as the meristem cap. The cytoplasm of the infected cells in the centre of the nodule becomes closely packed with bacteria which later on become branched and constitute the so-called 'bacterioids', and a group of these bacterioids form the nodule [Thornton, 1936]. Aeration helps the formation of the nodules, while passing nitrogen completely prevents nodulation [Virtanen and Hausen, 1936]. Wilson *et al.* [1936] have studied the effects of supplying additional CO_2 to clover and alfalfa on their nodule formation,

(iii) *Symbiosis between the plant and the bacteria*.—Evidence has been brought forward by a number of workers [Wilson, *et al.*, 1932 ; Barthel, 1921, 1926 ; Hopkins, 1929 ; Allison, 1929 ; Lohnis, 1930 ; Galestin, 1933 ; Pietz, 1937 ; Virtanen and Hausen, 1935, 2] that the host plant plays a more subtle role than mere furnishing room and board for the bacteria. It has been proved by these workers that the bacteria cannot fix nitrogen outside the host plant. Fred [1909] claims that certain amount of nitrogen is fixed by the bacteria even without the host plant, but this requires further confirmation. That the process of nitrogen fixation is the result of symbiotic relationship is further confirmed by the fact that the host plant by itself cannot fix nitrogen without the aid of the bacteria [Skallow, 1936 ; Guitschanoff, 1935]. The efficiency of nitrogen fixed is dependent more on the location than on the size of the number of the nodules [Rajagopalan, 1938].

The life of the bacteria in the host tissue has a definite bearing on its activity and efficiency of nitrogen fixation. A number of workers [Wumshik, 1925 ; Albrecht, 1920 ; Kalnins, 1938] have shown that the passage of the bacteria through the host plant results in increased activity. The beneficial effect on the host plant is the resultant of two factors, nitrogen fixation and inhibitory effect on plant growth due to growth of bacteria in the nodule. The effectiveness of the organism and its ability to aid the host plant are unrelated factors [Allen and Baldwin, 1931].

Erdman [1929] has reported that there is rapid translocation of the nitrogen fixed by the bacteria from the nodules to the seeds of the plant. Bond [1933] has reported that there is a quantitative relationship in the transfer of nitrogen between the bacteria and the host cells. He [1936] has further shown that more than 80 per cent of the nitrogen fixed by the nodule bacteria is liberated without appreciable delay in the host cytoplasm. The mechanism seems to be one of passive excretion by the bacteria in which the nitrogenous substances represent a phase of bacterial respiration rather than part of the process of bacterial synthesis.

Pietz [1937] has recorded the presence of the oxidation product of dihydroxy phenyl-alanine in the roots. This substance which changes in colour at specific η values is highly significant for its behaviour in the roots ; this to a large extent determines the growth of the bacterium. The dopa action alone does not, however, explain its role in the symbiosis mechanism.

Factors determining the host-bacteria equilibrium

The proper functioning of the bacteria within the host depends upon the maintenance of a physiological equilibrium between the host and the bacteria. Thornton and Rudolf [1936] have shown that the presence of NO_3 checks the infection of root hairs by protecting them against the normal action of bacterial secretions deforming them. This appears to be connected with the C : N balance in the root hairs. Secondly the cell walls of the distal meristematic cap cease to divide and form an increasingly thickened wall and thus isolate the bacterial tissue which later on shows symptoms of starvation finally becoming necrotic [Thornton, 1930, 1]. The equilibrium breaks down in old nodules even without the presence of NO_3 . This is due to the bacteria actually attacking the host tissue thereby becoming parasitic. Brenchley and

Thornton [1925] have shown that this state may be induced even in young nodules by certain changes in food supply, such as boron deficiency and failure of carbohydrate supply. It is probable that the change to parasitism is due to the bacteria being cut off from the supply of carbohydrate owing to the failure of the vascular strands.

(i) *Carbohydrates*.—An adequate and unhindered supply of carbohydrate seems to be essential for the healthy functioning of the nodule in the host tissue. Probably this supplies the energy for the fixation process [Ruffer, 1932]. Rippel and Krause [1934] have found that there is relation between carbohydrate and nodulation. Allison [1934] has observed that the nodules are located in the upper part of the root nearest the carbohydrate supply and when the supply becomes deficient they become dormant and in other cases they attack the host tissue to obtain food. In general, increased $p\text{CO}_2$ (partial pressure of CO_2) augments photosynthesis which in turn nodulation and nitrogen fixation. The effect is most pronounced between 0.03 and 0.01 CO_2 and is due to increased partial pressure and not the total amount present [Vita and Sandrinelli, 1935]. In the case of red clover, Georgi *et al.* [1933] have observed that higher $p\text{CO}_2$ is more effective. Allam [1931] has shown that light influences symbiosis between legume bacteria and host plant. Allison [1934, 1935] has suggested that high carbohydrate content is not essential for bacterial entrance but is necessary for root growth and that the bulk of the carbohydrate supply is used in growth and respiration of host tissue.

Certain amount of work has been carried out with a view to finding out the relative efficiencies of the different sugars as a source of supplying energy to *Rhizobium* cultures. The growth of *Rh. leguminosarum* in different sugars was in the following order: sucrose, lactose, glucose and dextrin [Madhok, 1935]. In agar media Reynolds and Werkman [1935] have found that sucrose was better than mannitol for the growth and longevity of *Rhizobia*. Georgi *et al.* [1933] have reported that addition of mannitol is not useful for nitrogen fixation; concentrations above 0.25 — 0.50 is detrimental, when inoculated with non-homologous species of bacteria and the plants die of nitrogen starvation even though carbohydrate is applied to them. No significant difference is observed in *Rh. meliloti* in the rate and extent of oxygen consumption in the media containing glucose, mannitol or sucrose. Arabinose was distinctly superior to other carbohydrates as a source of energy to *Rh. japonicum*.

Wilson [1935] and Walker and Anderson [1934] have shown that it is the carbohydrate-nitrogen relationship obtaining in the inoculated plants that is more important in symbiotic nitrogen fixation. Allison [1934] has found that the addition of nitrate alters the C : N ratio so that the carbohydrate is all used up for top growth and thus it inhibits nodulation. Honl [1930] has observed that the C : N relationship of the legume remains constant at various stages of growth while in non-legumes it varies.

More recently Allison and Ludwig [1938] have made a critical study of the available data in regard to legume nodule development in relation to available energy supplied and have concluded that the carbohydrate supply hypothesis accounts for the varying degrees of nodulation under different growth conditions, more adequately than the subsequently proposed carbohydrate-nitrogen hypothesis.

Weniger [1923] has suggested that the eventual energy requirement of nodule bacteria is so small as to be insignificant, the energy being hardly sufficient to support the life of bacteria—the requirements of non-symbiotic bacteria were five times as great. He has further suggested that the organisms are so constituted as to be able to transform energy incident to an exothermic nitrogen fixation process. Rippel and Poschenreider [1928] have found that soybean bacteria used enormous amounts of energy for nitrogen fixation, 34 calories in one experiment and 50 calories in another. Neal and Walker [1936] and Walker [1934] have shown that only one-third of the carbohydrate is oxidized to CO_2 and water and used for energy. It would appear that the carbohydrate is needed not only to feed the bacteria, but also the meristem of the host plant leading to the continual formation of young nodule tissue.

(ii) *Minerals and humic acid.*—Allison and Hoover [1935, 1] have shown that natural humic acid stimulates growth and oxygen consumption by *Rhizobia*; thus over a range of 0 to 600 p.p.m. of dry matter it was proportional to the quantity used. Small amounts of iron salts stimulated growth of *Rhizobia*; chloride was better than sulphate. The optimum concentration of iron was found to be 10 p.p.m. Smith [1907] has studied the effect of various iron salts in concentrations ranging from 0.00005 to 0.05 on *Asteralagus sinicus*. Stimulation was greatest with ferric malate and chloride, less with ferric salts sulphates, citrates, oxalate and tartrate and zero with other salts. Depression was greater with FeI_2 and $\text{Fe}(\text{NO}_3)_2$, less with FeCO_3 and least with ferric malate. Calcium adsorbed on colloidal clay transforms the abnormal forms into normal and effective nodules: the reason for the special activity of the adsorbed calcium is not understood. Ba in place of Ca produces the opposite effect [Albrecht *et al.*, 1937]. Itano and Matsuura [1936, 2] have recorded that titanium salts have specific morphological influence on the bacteria. Graham [1938] has studied magnesium as a factor in nitrogen fixation by soybeans and has shown that increase of Mg made it possible for the plant to make a more efficient use of Ca offered at a given level. The supply of exchangeable bases seem to be a limiting factor in legume growth and nitrogen fixation.

(iii) *H-ion concentration.*—The optimum pH for the growth of nodule bacteria in nutrient gelatin lies between 6.5 and 7.5 [Virtanen *et al.*, 1931, 1]. Maximum respiration and growth takes place near neutrality with constant activity between pH 6 and 7.8 [Thorne and Walker, 1935, 2]. In *Rhizobium meliloti* and *Japonicum* the optimum reaction for respiration is more alkaline than the optimum for growth [Thorne and Walker, 1936, 1]. The effect of soil acidity on nodule formation is not due to its effect on growth of roots of the plant but due to its effect on bacteria when it exists in the soil non-symbiotically [Karrakar, 1927].

(iv) *Accessory growth factors.*—Plant extracts greatly influence the growth of nodule bacteria [Allison, 1927]. Yeast and cane sugar contain the necessary growth factor for *Rhizobia* which can be extracted with absolute alcohol; no other substance tried was able to replace the factor present in yeast extract [Thorne and Walker, 1935, 1]. Potato extract stimulates the growth of *Rhizobia*, and asparagine to a less extent [Sarles and Reid, 1935]. Nilsson [1938] has shown that addition of materials like leguminous plants, molasses or

yeast is required for growth of *Bacillus radicola* in synthetic medium. Ver et al. [1936] have reported that reproduction and nitrogen fixation in nod bacteria are proportional to concentration of *bios* in the medium and that its presence the nodule bacteria can bind molecular nitrogen outside the h plant. Accessory substances present in bean nodule and yeast extract soluble in water and alcohol and chloroform and are non-dialysable [Itano and Matsuura, 1936, 1]. Sauerkraut has a more effective growth factor than yeast [Albrecht et al., 1937].

The factor present in sauerkraut is soluble in alcohol and dilute acetic acid, slightly soluble in methyl alcohol and insoluble in petroleum ether and pyridine. It is not absorbed on Fuller's earth and passes through colloid membrane but is destroyed by electro dialysis with complete chloride removal [Albrecht et al., 1937]. On electro dialysis of bean nodule most of the accessory substances were found in the cathodic chamber. There was no relation between accessory substances and nitrogen fixation [Itano and Matsuura, 1938]. The growth-promoting substance is an organic complex of relatively low molecular weight. It is probably not rhizopin auxin or inositol and in some respects it resembles *bios* [Clarke, 1936].

The important function of accessory growth substances of *Rhizobia* seems to be the provision of an initial H-donor which in turn lowers r_h and supplies a readily available initial source of energy. [Virtanen and Laine, 1935; Thorne and Walker, 1936, 2].

Recently, Laird and West [1938] have found that certain components of 'Wildiers bios' complex are capable of replacing the stimulatory action of yeast extract on strains of *Rh. trifoli* and this was proportional to the increase in urease activity produced by this factor. A number of compounds tested, including vitamin B₁, could not bring about this action. Nilsson et al. [1938] have shown that a second factor obtained by acid extraction from yeast in crystalline form is, in conjunction with vitamin B₁, highly active in promoting the growth of *Bacterium radicola*; the vitamin also causes increase in size of the bacterium and seems to favour formation of bacteroid branching. Steenberg [1938] has suggested that a second accessory factor is also necessary for the growth of *Rhizobia* and for which the name *Rhizobiosin* is proposed. West and Wilson [1939] have adduced evidence to show that the effect is due at least in part to the presence of thiamin and flavin in those products. Allison and Minor [1938] have reported that in the case of 19 strains of *Rhizobia* tested, the addition of coenzyme R to the medium is necessary in order to obtain appreciable growth; the need for this was equally evident when the organism was growing in combined nitrogen, thereby showing that it may not have a part to play in the fixation of nitrogen. The factor is of organic nature, although its chemical nature has not yet been determined. More recently Bjalfve et al. [1938] have shown that the growth effect of vitamin B₁ on *Rhizobium* holds good only for half of the strains isolated from clover and does not hold good for those found in peas, lupines and beans.

Allyn and Baldwin [1930] have shown that the legumes are very sensitive to slight changes in the oxidation-reduction character of the medium and it is likely that the beneficial effects of certain extracts may be due, in some instances, to their effect on this potential. Thus, ordinary mineral-salt media are too oxidized for optimum growth; a fair growth may take place even

uch unfavourable media if comparatively heavy inoculations are made, but oxidation-reduction potential must be adjusted very accurately in order, single cells, to initiate growth.

(v) *Alkaloids*.—The formation of bacterioids in *Bacillus radicum* is connected with the occurrence of plant bases, thus in the presence of caffeine it was possible to cause bacterioid formation in sterilized soils. Barthel [1926] reported that formation of bacterioids in the nodosities is dependent on presence of alkaloids in the roots. There is a relationship between the nitrogen utilized by the legume and the bases absorbed by it at different stages of growth [Stock and Rippel, 1929]. Caffeine influences the translocation of nodules into bacterioids but does not increase their capacity to fix nitrogen; the bacterioids probably cannot fix nitrogen of the air [Bazarewski, 1929; Berthelot, 1885]. In liquid cultures, caffeine in doses of 0.05–0.50 is an excellent stimulant, from 1.1 to 1.4 it is depressant, while above 1.5 it is toxic. Quinine and strychnine are more vigorous as stimulants between 0.05 and 0.10 and poisonous above 0.25. In solid cultures they are less vigorous [Assadrolí *et al.*, 1935]. Itano and Matsuura [1936, 1; 1937, 1938] have reported that alkaloids did not stimulate nodule bacteria, especially growth could not be observed with quinoline, and caffeine was most notable in producing large bacterioids which were associated with poor growth.

(vi) *Bacteriophage*.—Gerrotsa *et al.* [1923, 1924] and Hitchner [1930] have reported that the bacteriophage isolated from nodes of leguminous roots is probably active in dissolving bacteria. The lytic action is specific. The bacteriophage stands 55–65°C., passes through colloidal membrane and is 100 times more resistant to ultra-violet light than the bacteria. Grijns [1927] has found that the clover plant does not produce a bacteriophage and that under these conditions the presence or absence of the bacteriophage does not affect the growth of the plant. Demelon and Dumez [1938] have shown that the addition of bacteriophage filtrates, isolated from roots and nodules and heated to destroy the lytic agent, stimulated alfalfa growth.

(vii) *Nitrogen compounds*.—*Rh. meliloti* and *Rh. japonicum* produce ammonia from a number of amino acids tested; NO_3 is reduced to NO_2 and utilized by both species. There is a difference in the chemical action of various species on different nitrogen sources [Pohlman, 1931]. Amino acids are used directly by *Rhizobium* cultures and not through NH_3 stage, since the pH of the medium remained constant and NH_3 could not be detected [Virtanen *et al.*, 1931, 2]. The decrease of nodulation in presence of soil nitrogen salts is due to an inadequate supply of carbohydrate in the roots; the bacteria themselves play only a secondary role [Allison and Ludwig, 1934]. The influence of combined nitrogen on symbiotic nitrogen fixation depends on how it alters the C:N relationship in plants [Wilson and Wagner, 1937]. Thus the addition of NO_3 alters C:N ratio, so that the carbohydrate is all used up for top growth [Allison, 1934]. In *Rh. japonicum*, NO_3 -nitrogen was better than NH_3 -nitrogen [Neal and Walker, 1935]. Soluble nitrogen compounds which the plants utilize are not fixed by the bacteria [Georgi, *et al.*, 1933]. The growth of the nodules is limited by the limiting amounts of nitrogen fixed, the coefficient number of nodules and percentage of nitrogen being 0.57 ± 0.10 [Fred and Wilson, 1934].

Small amounts of glycine (0.1–0.5) result in partial or complete loss of infective ability of *Rhizobia* and *Phytomonas tumefaciens*, the loss being

complete after ten generations and permanent after thirty generations. Alanine, glycylglycine and dicynamide induce similar responses in several strains of *Phytomonas tumefaciens* [Longley *et al.*, 1937].

Products of metabolism of nodule bacteria

(i) *Slime*.—Slime is produced by the bacteria only when they fix nitrogen. It is produced in faintly alkaline and neutral media but not in acid media. Lipine produces slime in neutral media which rapidly become acid. The best medium for the production of slime contains 0.06 per cent asparagine, 0.01—0.2 per cent of alkali phosphate [Smith, 1907]. Hopkins *et al.* [1914] have observed that addition of nitrates to the medium increases gum production. Gum production is a normal process in the metabolism of the organism when purified gum is supplied to *Rhizobium*, it is not utilized as a source of carbohydrate [Anderson, 1933]. *Rh. radiculicola* produces gum from different carbonaceous materials, such as mono and disaccharides, dextrin, inulin, leavins, polyhydric alcohols containing 3, 5 and 6 carbon atoms and sodium salts of lactic, malonic and succinic acids. It produces no gum from salts of fatty acids, succinimide, malonamide and amino acids. The synthesis of gum is completely inhibited by high concentrations (5-10 per cent), optimum being 1-2 per cent. It is a polysaccharide containing glucuronic acid [Cooper and Peterson, 1937]. The gum produced by cross inoculation groups is precipitated by acetone and is free from nitrogen. The carbon content varies from 36.4 to 40.6 per cent; on hydrolysis it yields glucose and not pentoses; it contains 4.1—25.3 per cent uronic acid and the complex is probably glucuronic acid [Greaves and Anderson, 1914]. Condition for the production of gum is similar to that of *Azotobacter* [Cooper and Peterson, 1937]. More recently Cooper *et al.* [1938] have shown that there is a close similarity in the polysaccharides of *Rh. radiculicola* and those of *Azotobacter* and have suggested that these belong to the same class as the polysaccharides of the pneumococcus types II and III.

Georgi and Wilson [1933] have suggested that the gum may be an intermediate step in the oxidation of carbohydrate into CO_2 . The evidence for this view is that if the organism is grown in the presence of a limited quantity of oxygen, so that respiration is arrested after a few days' growth, only 50 per cent of the glucose which disappears could be accounted for as CO_2 , whereas in presence of sufficient oxygen in the medium the amount of glucose carbon which disappeared as CO_2 rose to 70-80 per cent.

(ii) *Proteins*.—The properties of protein of legumes being very similar to those of casein, Rukuzin and Pekrskaya [1920] have suggested the name vegetable casein for it. From a study of the protein make-up of roots and tubercles of *Vicia faba*, it is concluded that the co-presence of free amino acids and reducing substances in considerable quantity in the bacterial tissue of tubercles indicates the existence of a direct relationship between these substances and the synthesis of proteins [Parisi *et al.*, 1926]. The chemical composition of nodular tissue is not different from that of the other part of the plant. The dry matter of nodule bacteria consists chiefly of carbohydrates; it contains 52.8—54.6 per cent carbon, 4.4—4.9 per cent of nitrogen, 11.4—22.6 per cent fat [Carrol, 1934, 1]. 20 per cent of the nitrogen of the nodules is arginine.

soluble portion of fresh nodular tissue contains much arginine and is probably the basic amino N previously thought to be of importance in symbiotic nitrogen fixation. There is no difference in the composition between different species. The total nitrogen in heavy gum producers is 2 per cent, while that of alfalfa is 8.4 per cent [Umbreit and Burris, 1938]. The nitrogen content of bacterial tissue increases with the age of the culture and with decrease in C : ratio [Rajagopalan, 1938].

(iii) *Fermentation products*.—Acids are produced by fermentation of sugars with alfalfa bacteria. 5.5 per cent of the weight of sugar was fermented into acids with sucrose, and 7.3 per cent with lactose. Anderson *et al.* [1928] have detected pyruvic acid in *Rhizobium* culture. The power to ferment sugars by *Astragalus sinicus* (Genge) varied in the order arabinose, glucose, galactose, mannose, fructose, sucrose, mannitol, lactose, maltose, raffinose and dextrin. Nitrogen source is not necessary for growth, but is necessary for fermentation [Matsuura, 1935]. During fermentation of sugars by groundnut nodule organism acetic acid, ethyl alcohol, traces of acetaldehyde, tartaric acid and carbon dioxide were detected. In nitrate medium 10 per cent of the carbon supplied is used for cell formation, 21 per cent is oxidized to CO_2 and the rest converted into other by-products [Rajagopalan, 1938]. The fermentation is of the pyruvic acid type [Virtanen *et al.* 1933, 2]. Burney *et al.* [1935] have suggested that pantothenic acid produced by *B. meliloti* plays a part in the carbohydrate anabolism of the plant.

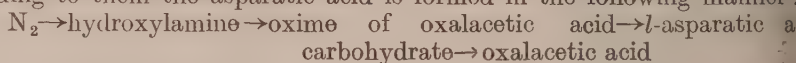
biochemical mechanism of symbiotic nitrogen fixation

(i) *The first intermediate product in symbiotic nitrogen fixation*.—With a view to finding out the first chemical step in fixation of nitrogen in the symbiotic system, several workers have attempted to demonstrate the first intermediate product formed during the process. Notable among these workers are Winogradsky, Virtanen and his associates, and Orcutt.

According to Winogradsky [1933, 1938] and Winogradsky & Winogradsky [1936] the liberation of ammonia from root nodules is the result of nitrogen fixation and not ammonification because (a) maximum production of NH_3 takes place on the second day followed by a decrease, (b) the effectiveness of antiseptics and anaesthetics, (c) evolution of ammonia from dried and powdered nodules at 40—50°C., and (d) the fact that neither the roots of maize nor legume roots from nodules were capable of forming ammonia. Since Winogradsky failed to demonstrate that the nodules in his experiments were fixing nitrogen and since all previous experiments with detached nodules under conditions similar to those used by him, have been negative with respect to fixation, his conclusion that ammonia is the first product is hardly acceptable.

Virtanen and his colleagues [1936] have brought forward evidence to show that aspartic acid, which is excreted along with lysine, is the primary product of nitrogen fixation. Virtanen and Laine [1936; Virtanen, 1936, 2; Virtanen and Laine, 1938, 1] have detected small amounts of NO_3 and NH_2OH in addition to aspartic acid; they have suggested that aspartic acid may be formed from hydroxylamine and oxalacetic acid and the NO_3 may arise from oximes originally present in the cultures. They [1937] have further shown that the root nodule bacteria split off CO_2 from aspartic acid and thus produce alanine.

By *in vitro* experiments it has been possible to demonstrate that when pyruvic acid and l-asparatic acid are added to crushed pea plants alanine is formed. This transfer of NH_2 from asparatic acid to keto acid is similar to that occurring in animal tissues. They have further suggested that the nitrogen is probably converted by an unknown intermediate into hydroxylamine which probably reacts with oxalacetic acid to form oxime which is later reduced to give l-asparatic acid [Virtanen, 1938]. Virtanen [1937, 2] has demonstrated that the legume bacteria split off one of the carboxyl groups from l-asparatic acid forming β -alanine. The reaction is almost quantitative at pH 7.0 and is accomplished by living bacteria. In support of this, they have also demonstrated nitrogen fixation by excised root nodules in oxalacetic acid medium. According to them the asparatic acid is formed in the following manner:



More recently, Virtanen *et al.* [1938] have adduced evidence to show that the legume bacteria contain two different amino acid carboxylases, aspartate decarboxylase and glutamic decarboxylase. Wilson [1939, 2] has, however, failed to confirm Virtanen's findings; he was unable to accomplish nitrogen fixation by excised root nodules; he has also failed to detect the presence of oxalacetic acid in the medium by the aniline manometric method of Osterhout.

From a study of nitrogen compounds in different parts of the legume supplied with free and fixed nitrogen, Orcutt [1937] has suggested that the only fraction that appears to offer possible significance in the fixation process is the basic amino fraction. Umbreit and Burris [1938] have confirmed this and have further shown that this fraction has an unusually high content of substance which gave ammonia on hydrolysis with alkali. This has been tentatively identified as arginine. The conclusion drawn from these composition studies can, however, be regarded only as suggestive.

It is evident from the foregoing that our present knowledge of the final chemical step in symbiotic nitrogen fixation is still meagre for want of exact and reproducible data.

(ii) *Excretion of nitrogen compounds from root nodules.*—The nitrogen compounds excreted by nodules are mainly amino acids; no NO_2 or NH_3 is present, but small amounts of amides and volatile bases, probably amines, are found in the dialysed portion [Virtanen *et al.*, 1933, 1]. 87—98 per cent of the total nitrogen excreted is amino nitrogen; asparatic acid accounts for 50 per cent while the other half can be precipitated by phosphotungstic acid but is not arginine, cystine, histidine or any aromatic amino acids [Virtanen and Laine, 1935]; probably it is lysine [Virtanen *et al.*, 1936]. The major part of the amino acid excreted from quartz cultures of inoculated peas before flowering is l-asparatic acid nitrogen, while if the peas are almost mature, chiefly β -alanine is found in the cultures [Virtanen and Laine, 1937]. Nitrogen excretion from inoculated plants takes place from the bacteria inside the nodules and not from the roots [Virtanen *et al.*, 1937].

Air supply results in an increased rate of nitrogen excretion [Virtanen and Hausen, 1934, 1935, 1]. Excretion of nitrogen is highest in young roots [Virtanen and Hausen, 1935, 2] and the rate of excretion is maximum before blooming [Virtanen *et al.*, 1936]. The nitrogen compounds, especially

aspartic acid and lysine, are excreted only slightly in water, more so if there is sand or clay in the water and still more so in soils in which non-legumes are growing [Virtanen, 1936, 1]. Virtanen and Hausen [1936] have shown, in fact, that direct contact of the roots and the nodules with solid particles is necessary for nitrogen excretion.

Virtanen *et al.* [1937] have shown that non-legumes in association with legumes excrete considerable quantities of nitrogen. In sterile cultures of lupines amino acids were excreted in considerable quantity when the plant was young [Kova and Andreev, 1938]. Under favourable conditions of photosynthesis there is no or poor excretion of nitrogen. Lowering of temperature and shading increase excretion. Ammonia, nitrate and cyanamide lowered nitrogen excretion in white clover [Wilson and Wyss, 1937]. The extent of nitrogen excretion depends largely on the strain of the organism and the quantity of nitrogen available [Virtanen *et al.*, 1937].

The legumes receive their nitrogen supply from the nodules in the form of amino acids which are the products of nitrogen fixation and not protein breakdown [Virtanen *et al.*, 1937]. Virtanen *et al.* [1933, 1] have shown that aspartic acid is an excellent source for leguminous plants but is entirely unavailable for cereal plants. The excreted nitrogen is utilized by non-legumes, such as beets, and wheat used 50 per cent while potatoes 90 per cent; with increase in excretion, peas utilize only a fraction of the nitrogen fixed by the nodules [Virtanen *et al.*, 1937]. The excreted nitrogen is also utilized by barley and other plants grown by the side of the legumes; barley utilizes lysine in preference to aspartic acid [Virtanen *et al.*, 1936].

Excretion of nitrogen is not universally obtained under experimental conditions which are apparently identical [Wilson, 1937]. Thus Bond [1937, 1938] has reported that no nitrogen was excreted in sand cultures by soyabean and broad bean, and small amounts only with peas. Perhaps the failure to detect excretion is due to use of coarse sand which lacks absorptive capacity [Virtanen, 1937, 1]. By reducing the exposure to sunlight of pea plants previously exposed to normal daylight, Strong and Thrumble [1939] have confirmed excretion of nitrogen by roots. Recently, Sharper [1939] has described a simple technique by which he has detected excretion of nitrogen in lucerne plants inoculated with *Rh. meliloti*.

The origin of the excreted nitrogen is not, however, very clear. Virtanen and Hausen [1935, 2] have reported that it is not due to mechanical injury but that it takes place even in agar cultures. It represents the primary product of nitrogen fixation and not decomposition products of protein, since no excretion takes place in uninoculated plants [Virtanen *et al.*, 1936]. On the other hand, Wilson and Burton [1938] have reported that excretion does not always accompany fixation and it appears to be the exception rather than the rule in greenhouse studies.

(iii) *Respiration studies.*—Barthel [1932] has carried out experiments with different strains of *Bact. radicicola* in culture solution under reduced oxygen pressures and has shown that by an amount of only 0.5 per cent of oxygen in a mixture of nitrogen and oxygen, or of hydrogen and oxygen, the growth of the bacteria amounted to about 25 per cent of the growth in the controls held under normal aeration.

The rate of respiration for nodule bacteria is considerably higher than that of other bacteria. From a study of its respiration using various media, Georgi and Wilson [1933] have classified the organisms as obligate aerobes. The organism grows well even under low oxygen tensions (less than 0.03 atm.). In low oxygen tensions glycolysis rather than butyric fermentation takes place [Virtanen *et al.*, 1934]. During the dissimilation process the R. Q. (the respiratory quotient) reaches 1.18 after which it decreases and remains constant, probably this marks the change from carbohydrate to protein metabolism and a large part of the oxygen thus not accounted for can be used for the formation of new cell tissue.

The respiratory quotient of nodule bacteria varies from 0.90 to 1.10. In the majority of bacteria it is slightly greater than 1.0 [Wilson and Peterson, 1933]. In glucose yeast extract medium the R. Q. was consistently less than 1.0 [0.85—0.96]. With NaNO_3 in glucose the R. Q. increased to 1.07—1.17, NO_3 being used as an H acceptor in place of O_2 . In nitrogen medium the R. Q. was less than 1.0 [Anderson and Walker, 1933]. The R. Q. in *Rh. trifolii* is higher than in other species.

The utilization of carbon by *Rhizobia* decreases with increase in concentration of sugar; with 1 per cent glucose all carbon was used up, while with 2 per cent only 50—70 per cent was used up [Wilson and Peterson, 1933]. The rate of oxygen consumption increases with increased oxygen pressure. With decrease in oxygen concentration, consumption of oxygen was represented in *trifolii* but not in *meliloti* and *lupini*. Growth of nodule bacteria in an atmosphere containing 0.5 per cent oxygen (in H_2 or N_2) was approximately 25 per cent that occurring under normal conditions [Anderson, 1933]. Respiration in *Rh. trifolii* is pronounced in an atmosphere in which CO_2 is increased to 0.05 per cent [Konishi *et al.*, 1936].

A respiratory co-enzyme, which is essential primarily for respiration and indirectly for growth, is present in relatively high concentrations in yeast, cane molasses, humic acid and commercial egg albumin. Yeast extracts increased the rate and extent of oxygen consumption in all strains of *Rhizobia* [Walker and Anderson, 1933]. Half the maximum growth is obtained within 16—20 p.p.m. of these extracts in the synthetic medium [Allison and Hoover, 1934]. Such stimulation could not be traced to the presence of nitrogen, carbohydrate, vitamins or salts in these extracts. Neither the nitrogen nor sugar requirements of *Rh. trifolii* are specific [Allison and Hoover, 1934]. Co-enzyme R. increases bacterial respiration two to five fold within an hour and the rate of growth of the organism is increased 20—30 times; but it has no function in nitrogen fixation. Thorne and Walker [1934] have, however, suggested that the stimulation obtained by the above materials can be satisfactorily explained on the basis of their nutritional value and were unable to substantiate the assumption of a co-enzyme for respiration. The amount of oxygen consumed was greater in yeast extract medium than any other medium.

Wilson [1938] has studied different substrates as H-donators with oxygen and methylene blue in *Rhizobium* cultures. Among sugars, glucose and arabinose were the best. With polyhydric alcohols the cultures were active towards oxygen but not methylene blue (with the exception of sorbitol); probably in these oxidations an aldose is formed as an intermediate product. Among the organic acids the highest respiration occurred with fumarates.

accinates. From a study of the oxidation of different substrates in *Rh. tri-
dii* and *meliloti* cultures, Konishi and Kawamura [1938] have shown that
different kinds of oxygenases are present in the cells of nodule bacteria.
Catalase was more positive in bacterial culture from clover, alfalfa, genge,
pine and soybean. Peroxidase was more pronounced than catalase, espe-
cially in clover and soybean.

From a study of the physiological effects of various materials, such as
yeast extract, Thorne and Walker [1936, 3, 4] have concluded that the role
of the H-donor in this organism appears to be twofold; firstly, it tends to
lower the oxidation-reduction potentials and secondly, it furnishes the orga-
nism with a rapidly available initial source of energy.

(iv) *Enzyme systems in symbiotic bacteria*.—The study of nitrogen-fixing
enzyme system present in the symbiotic bacteria is difficult because of the two
component nature (plant and bacteria) of the system responsible for nitrogen
fixation. Nevertheless, a beginning has been made in this direction by Wilson
and his colleagues. They have adopted the classical Warburg manometric
method that is generally used for the study of enzyme system concerned with
respiration of intact cells. By using this technique they could study the
various factors responsible for nitrogen fixation in the symbiotic system.
Rigorous statistical treatment of the mass of data obtained from carefully
controlled experiments has been employed to determine the significance of
their results.

It is well known that the rate of reaction in an enzyme system depends on
the concentration of substrates. Using red clover plants Wilson [1936; 1939,
2] has studied the dependence of nitrogen-fixing reaction on the pN_2 in the
atmosphere. The maximum fixation was obtained when the pN_2 reached
0.15—0.20 atmospheres, and there was a significant decrease in the quantity
of nitrogen fixed only after the pN_2 was reduced to 0.1 atmosphere. On the
basis of available data the Michaelis constant, K_{N_2} (the thermodynamic dis-
sociation constant of the nitrogen-fixing reaction) for symbiotic nitrogen fixa-
tion appears to be 0.05 ± 0.005 atmosphere.

It would be of interest to study the influence of pO_2 on nitrogen fixation
in view of the fact that molecular oxygen is involved in some of the mechanisms
postulated in symbiotic fixation. From a study of the influence of oxygen
under various pressures on the assimilation of nitrogen in the free and fixed
state, Wilson and Fred [1937], Wilson and Bond [1936] and Thorne and Burris
[1938] have shown that molecular oxygen does not play any direct role in the
mechanism of nitrogen fixation. However, it may indirectly influence the rate
of fixation through effects on the carbohydrate relationship in the host plant.

In the course of his studies on the influence of different nitrogen pressures
Wilson found that the pN_2 function (the relation between nitrogen fixation and
 pN_2) in presence of H_2 differs greatly from that in the absence of the gas. He
further showed that the uptake of combined nitrogen was dependent on the pre-
sence of hydrogen in the atmosphere. Wilson and Umbreit [1937] have re-
cently examined this question and have adduced evidence from different ex-
periments to show that hydrogen itself rather than the accompanying impurity
is the inhibitory agent. Their findings would suggest that hydrogen may be a
specific inhibitor for the symbiotic nitrogen fixation process. This observation
is interesting in view of the fact that several workers, notably Stephenson, have

considered hydrogen as a biologically active substance but is quite inert towards non-symbiotic nitrogen fixation reaction brought about by *Azotobacter* [Bull 1934].

Among the other common enzyme inhibitors concerned with oxidation-reduction reactions in the symbiotic system Wilson [1939, 2] has shown that only CO and H_2S possess a specific inhibitory effect on symbiotic nitrogen fixation by red clover plants. The effect of CO is more interesting because the small concentrations required: nitrogen fixation in red clover practically ceases when the concentration of CO in the atmosphere reaches 0.1 per cent.

Among the other enzymes present in the symbiotic system evidence has been obtained to show that the bacterial cells contain gelatinase, catalase, deaminase, carboxylase, tyrosinase, urease, oxidase, peroxidase and various sugar-splitting enzymes [Rajagopalan, 1938]. Eckhardt *et al.* [1931] have observed that *Rh. lupini* produces slight amount of tyrosinase. Almon and Fred [1933] have shown that root nodule bacteria of some cross inoculation groups, notably the bean, alfalfa and soybean groups, showed a higher percentage of cultures producing tyrosinase than did others. The cells contain very little of proteases, toluenated *Rhizobium* culture produce very little proteolysis in vegetable proteins and none at all in cell proteins.

NITROGEN FIXATION BY HIGHER FORMS OF LIFE

In recent years increasing evidence has been obtained that the fixation of atmospheric nitrogen is also manifested in some higher forms of plant life. Certain species of algae, yeast, fungi, germinating seeds of legumes and a number of other plant cells have been found to fix nitrogen.

Algae

The investigations of Kruger and Schneidwind [1900] showed that there is no assimilation of free nitrogen in algae. They suggested that probably algae under natural conditions are favourable to the growth of nitrogen-fixing bacteria. Schramm [1914] also found that none of the seven species he experimented with were able to fix atmospheric nitrogen. Wann [1920] has, however, reported that the seven species of grass green algae which were grown in pure cultures in Kjeldahl flasks in mineral nutrient agar containing known amounts of nitrogen (ammonium nitrate and calcium nitrate but not urea, glycocoll, asparagin and ammonium sulphate) fix in presence of glucose 4-13 mg. of nitrogen in five to seven months. Bristol and Page [1923] have, however, failed to confirm Wann's findings. They conducted experiments with four species of green algae in pure culture and they could not find any fixation of nitrogen. They have criticised Wann's methods of analysis as being unreliable.

Moore and Webster [1920] came to the conclusion that unicellular algae can grow and synthesise protein in the absence of all other sources of nitrogen except the elementary nitrogen of the atmosphere, provided CO_2 is present in the medium. Joshi [1928] has reported that the algae in the soil fix atmospheric nitrogen. Drewes [1928] has observed that *Anabaena variables* and *Anabaena* spp. fix 2-3 mg. of nitrogen in 250 c.c. medium in two months. Allison and Morris [1930] have observed that while the species of green algae tested did not fix nitrogen, the blue green algae isolated from soil in pure culture fixed atmospheric nitrogen in presence of light. They [1932] have also shown that the blue green algae *Anabaena variables* fixed appreciable amounts

of atmospheric nitrogen in presence of light and 1 per cent CO_2 ; soluble nitrogen compounds are produced in the medium. Allison and Hoover [1935, 2] have found that pure cultures of *Nostoc* isolated from soil fixed nitrogen. The dried organism contained 4.6 per cent of nitrogen. Cultures fix 10—20 mg. of nitrogen per 100 c.c. in 50—60 days. In presence of light there was increased growth and nitrogen fixation by the organisms, while in total darkness, nitrogen fixation and chlorophyll formation took place when glucose was supplied to them. They have further shown that the rate of growth and fixation in *Nostoc mucosarum* is 10—20 times greater than that reported for other nitrogen-fixing blue green algae. The quantities of nitrogen fixed are as high as 10 mg. in 45 days and 18 mg. in 85 days per c.c. of a medium containing no carbohydrates. Calcium and strontium are not essential for growth in presence of combined nitrogen, but in nitrogen-free medium, nitrogen fixation is retarded by their absence. Boron and manganese have no effect on nitrogen fixation [Allison and Hoover, 1937]. De [1936] first suggested that fixation of nitrogen in waterlogged soils is an algal process.

More recently, Fritsch and De [1938] have reported that pure cultures of blue green algae *Anabaena* found in Indian rice fields have the property of fixing nitrogen from the air; three species of the organism cultured in nitrogen-free solutions were able to fix 2—5 mg. of nitrogen per 1,000 c.c. medium in about two months. They have claimed that this is the first conclusive proof of the ability of a blue green algae to do so. By repeated sub-culturing on sterilized silica gel plates, De [1939] has isolated pure (bacteria-free) cultures of three species of *Anabaena* and *Phormidium foveolarum*. The *Anabaena* cultures have been found to fix considerable amounts of nitrogen in nitrogen-free medium and soil; small amounts of soil extract in the medium stimulated nitrogen fixation. *Phormidium foveolarum*, on the other hand, afforded no evidence of nitrogen fixation. He has also found that a considerable part of the nitrogen fixed remains in the external medium in an organic form. The author has concluded that algae are the chief agents of nitrogen fixation in the rice fields.

Yeasts, fungi and actinomycetes

Lipman [1910] has shown that certain species of yeasts and pseudo-yeasts have the power of nitrogen fixation in tap water solutions containing dextrose. Kossowicz [1913, 1914] has reported that 5—7 mg. of nitrogen was fixed by *Saccharomyces monilia*, *candida* and *Oidium lactis* in 500 c.c. of non-nitrogenous medium. Fulmer [1923] has shown that *Saccharomyces cerevisiae* will grow in an apparently good state of nutrition using atmospheric nitrogen as the sole source of nitrogen and has suggested that the benefit accruing from the aeration of yeast is as much due to the addition of nitrogen as of oxygen. He has found that fixation of nitrogen by yeasts at 30° is a function of pH, there being two optimal concentrations, the one at pH 6.0 and the other at 7.9, the latter being more potent. The failure to observe any fixation in yeast by different workers has been explained by Fulmer and Christensen [1925] as being due to the time element, and the presence of ring nitrogen compounds which are converted in the early stages into forms not determined by the Kjeldahl method. Christensen [1928] has shown that there is probably more fixation of nitrogen in pure cultures of *Saccharomyces cerevisiae* than what any available method of determination indicates.

Two types of fungi are generally recognized to be of significance in fixation of atmospheric nitrogen, the free-living fungi and the symbiotic group known as *mycorrhiza*, which live in the roots of certain higher plants.

Froenlich [1907] found that several strains of fungi fixed from 1.1—8.9 mg. of nitrogen, the amount of nitrogen fixed per gram of dextrose being —8.9. Stabel [1911] has reported that 9 of the 54 strains studied showed fixation of nitrogen. Schober [1930] has found that all the six strains of *Aspergillus* fixed up to 4 mg. of nitrogen in 100 c.c. medium containing per cent sugar. Kadelbach [1931] and Schroder [1931] have, however, failed to confirm this observation. Chambers [1916] has adduced evidence to show that no nitrogen is fixed by the free-living fungi, *Aspergillus niger* and *Penicillium glaucum*. Waterman [1913], Goddard [1913] and Duggar and Davidson [1916] have also obtained negative evidence in regard to nitrogen fixation by free-living fungi. More recently, Allison *et al.* [1934] have concluded that free-living fungi and actinomyces do not fix nitrogen.

There is strong evidence that certain micorhizal fungi can use atmospheric nitrogen when growing in the roots of plants. Rayner [1922] showed that certain strains of *Phoma*, isolated from the roots of ericaceous plants, utilize atmospheric nitrogen. He has also shown that seedlings of *Calluna vulgaris* in pure culture thrive in rooting media deprived of nitrogen. Jones and Smith [1928] obtained similar results and further demonstrated that when the pure micorhizal fungus, *C. vulgaris* grown in presence of molecular nitrogen uses large amounts of glucose with increase of nitrogen in the medium. Esker [1938] has suggested that lichens represent symbiosis of three organisms—nitrogen fixing bacteria in addition to fungus and algae, and that the bacteria present are the intragonidial wart-like swellings found in them. In view of the conflicting evidence, it is still doubtful whether the yeasts, fungi and actinomyces are of any importance in nitrogen fixation.

Germinating seeds

Vita [1932, 1] has obtained evidence to show that germinating seeds of legumes have the power of assimilating atmospheric nitrogen. When seeds of pea, lupine or horse bean are germinated in an atmosphere containing CO_2 in presence of alkaloids or even dilute solutions of salts, they absorb nitrogen for their development [Vita, 1932, 2]. Vita and Sandrinelli [1932, 1933, 1935] have studied the various factors that influence this fixation of nitrogen. They have shown that the amount of elementary nitrogen utilized is dependent on the nature and amount of salts, CO_2 , O_2 concentration, temperature and conditions of illumination. They have also observed that in pea and lupine seeds, there is a general relation between nitrogen-fixing and oxidizing power of the seeds. Sugars and some alkaloids like strychnine nitrate and caffeine have marked negative effect. Vita [1935] has found that later in the period of germination the gain in nitrogen partially disappeared. Using lupine and pea seeds, Haritantis [1934] has confirmed these findings. Vita has also postulated that during germination the seeds elaborate an enzyme, azoligase, which is capable of fixing atmospheric nitrogen. The added compounds stimulated its production which resulted in fixation of free nitrogen. The subsequent drop in the nitrogen content of the seedlings has been explained as being due to the action of another enzyme which liberated the nitrogen. Sadasiva

and Sreenivasan [1937] have also observed that there is a progressive increase in nitrogen assimilation in germinating seeds and have suggested that the seeds, independent of any organism, fix atmospheric nitrogen.

Recently, Wilson [1939, 2] has critically examined the available data in regard to the nitrogen fixation by germinating seeds and has questioned the validity of the conclusions arrived at by these investigators. He has shown that at the apparent increase in nitrogen which Vita has obtained is due to the inadequacy of the special analytical method used in her studies. The official Kjeldahl method (without addition of water) does not yield all the nitrogen in seeds. Smith and Wilson [1935] have shown that when a suitable method of analysis was used peas which were germinated under identical conditions prescribed by Vita did not show any gain in nitrogen on germination. Their conclusions are supported by data obtained by the gasometric method wherein so the nitrogen fixation was not apparent beyond the experimental error.

In view of the conflicting evidence it is difficult to draw any conclusion regarding nitrogen fixation by leguminous seeds on germination. Any positive evidence in this direction would no doubt be of much significance in studying the mechanism of symbiotic nitrogen fixation by legumes.

Fixation in other plant cells

Several workers have reported from time to time that different parts of higher plants exhibit the power of fixing atmospheric nitrogen, either by themselves or by their association with the bacteria present in them. But evidence so far obtained is still inadequate to draw any definite conclusion regarding the relative importance of these as nitrogen fixers.

Lipman and Taylor [1924] have shown that wheat and barley in culture solutions with and without nitric nitrogen fix atmospheric nitrogen without bacterial intervention. Whitley [1923] has also observed that higher plants have got the capacity of fixing atmospheric nitrogen. Moore obtained similar results with other plants. Burk [1927] has shown that the dwarf variety *Pisum sativum* lost enough nitrogen through excretion in culture solutions to hide any fixation. Brown [1933] has reported that perennial rye-grass meets some of its nitrogen requirements from the atmosphere, especially when nitrogen in the combined form is absent from the medium.

Symbiosis between bacteria and leaves of certain plants was observed in the case of *Pavetta* [Faber, 1912, 1914], *Andrisia crispa* [Miehe, 1914, 1911, 1916] and *Kraussia* [Georgvitch, 1916]. Knots are formed at the place of penetration of the microbes into the tissues of the plant. It is claimed that the bacteria bringing about this transformation can also fix nitrogen when not working symbiotically with the plants. The amount of nitrogen thus fixed may be so considerable that in India Pavetta plants are used as green manure. Rao [1933] has shown that the leaf nodules of *Chomelia asiatica* contain colonies of aerobic nitrogen-fixing bacteria. The symbiosis is developed to a greater extent than leguminosae and is of a hereditary character, the plant being unable to grow in the absence of the bacteria. Cauda [1919] has suggested that *Acetabularia cruciferae* isolated from various cruciferous plants is found to fix nitrogen, especially when cultured in liquid medium with an excess of calcium carbonate and deficient in nitrogen. The amount of nitrogen fixed is equal to that obtained by *Azotobacter*.

Henry [1904] has reported that dead leaves of various trees fix considerable amounts of atmospheric nitrogen. From experiments with plants grown in nitrogen-free atmosphere Kovessi [1912] has concluded that the plant hair and phanerogams cannot fix atmospheric nitrogen. Sahasrabudhe [1935] reported that nitrogen fixation in rice soils is increased by the presence of growing roots of plants. He [1936] has adduced further evidence to show that nitrogen-fixing organisms are active in the presence of rice roots which are good hosts for them. Several investigators [Joshi, 1928; Truffaut and Bezssonov, 1925, 2; Caron, 1923] have also reported fixation of nitrogen by higher plants (barley and corn roots) in presence of various species of bacteria but no nodule is produced. Oes [1913] has reported that the floating fern, *Azolla*, can grow in nitrogen-free medium and fix atmospheric nitrogen. He further showed that the blue green algae in symbiosis with it account for the nitrogen fixation.

AGRICULTURAL IMPORTANCE OF NITROGEN-FIXING ORGANISMS

The practical importance of biological nitrogen fixation in agriculture cannot be overestimated. The nitrogen-fixing organisms constitute perhaps the most important factor in maintaining the store of nitrogen in the soil. By proper control of the activities of these organisms it is possible to use them as natural agencies—plant and soil bacteria—to supply the nitrogen requirements in the soil for plant growth and crop production.

Role of the different forms in nature

(i) *Azotobacter*.—The occurrence of *Azotobacter* in the soil is conditioned by three factors, viz. the soil reaction, the soil complex and the available phosphorus content [Gainey, 1925; Wilson and Wilson, 1933; Martin *et al.* 1937]. Soils having pH value not lower than 6, and available phosphorus, particularly in certain proportion to the carbonate content, generally show a good culture of *Azotobacter*, containing 300 per c.c. [Beijerinck, 1921].

Although *Azotobacter* is more potent in tropical climates, it is present in almost all soils of the world. Its occurrence has been demonstrated in many Java soils, in all soils in India, in half of the Polish soils and in about 33 per cent of the cultivated soils of Japan [Hutchinson, 1915; Yamagata and Itano, 1923]. Greaves [1918] has observed that it is very widely distributed in Utah and Danish soils. Dianowa and Woroschilova [1931] have, however, reported that it is completely absent in Finnish soils, even in those that are well buffered and supplied with CaCO_3 .

The efficiency to fix nitrogen by *Azotobacter* varies with different strains and is markedly affected by seasonal fluctuations [Walton 1915; Vandecasteele 1938]. Krzenieniewski [1909] has observed that the other soil bacteria have little influence on its activity. Several workers [Konishi and Tsuge, 1933; Walton 1915; Vandecasteele and Anderson, 1934; Oes, 1913; Bortels, 1931; Ehrenberg, 1910], have observed that the applications of suitable chemical fertilizers and compounds of molybdenum and vanadium and fertilizers, such as basic slag, lime, phosphorus and carbohydrates, increase the number of *Azotobacter* and enhance their activity in the soil. The action of basic fertilizers is not only due to neutralization but also due to the presence of iron and

manganese in them which act as stimulatory agents [Mockeridge, 1914]. Generally speaking nitrogenous compounds depress nitrogen fixation in the soil [Hills, 1917]. Schneider [1931] has, however, observed that the application of NaNO_3 and urea favours the growth of *Azotobacter chroococcum*, while a continuous dressing of ammonium sulphate retards its development. Baumantel and Simon [1929] have suggested that the unfavourable effect of much calcium and water in the soil on *Azotobacter* is presumably due to the physiological action of $\text{Ca}(\text{HCO}_3)_2$ and not due to flocculation of soil colloids.

Our knowledge of *Clostridium* in relation to soil fertility is meagre. Probably these organisms fix nitrogen in the deeper layers of the soil under anaerobic conditions and give off their nitrogen to the plants.

It is fairly certain that non-symbiotic or free-living bacteria considered as a unit, play a definite part in maintaining the supply of nitrogen in the soil by fixing nitrogen from the air. Carter and Greaves [1928], as a result of vegetation experiments lasting over several years, have recorded annual gains of 5 lb. of nitrogen per acre-foot. The estimates made by Hall [1912] as also his later data from Rothamstead would point out to the same conclusion. Several Russian investigators, prominent among these being Kostichev *et al.* [1926], have also stressed the importance of non-symbiotic fixation in the soil. Wilson and Ali [1922] have reported 100 per cent increase in total nitrogen contents of soils in the district of Punjab (India) due to bacterial fixation.

The relative importance of the various non-symbiotic bacteria responsible for the increase of soil nitrogen is not, however, well understood. Most of the European investigators have attributed more importance to the aerobic organisms, especially the *Azotobacter* group. On the other hand, many of the American investigators consider that *Azotobacter* is not so important as the other non-symbiotic organisms. Bonazzi [1915] has shown that there is not yet conclusive proof that *Azotobacter* is of any value under field conditions as a nitrogen gatherer. Waksman [1931] has also reached the same conclusion.

(ii) *Legume bacteria*.—Riede and Bucherer [1939] have found that the soybean nodule bacteria markedly enhance the vegetative and reproductive growth of the plants in nitrogen-poor soil.

Application of nitrogenous fertilizers has a definite influence on nitrogen fixation by nodule bacteria. It has been found that large amounts of nitrate and ammonium sulphate, whether added or accumulated, are injurious to nodule formation in alfalfa, vetch and clover and this in turn on nitrogen fixation [Fred and Graul, 1916]. Albrecht [1920] has reported that nitrogen fixation by *Pseudomonas radiculicola* will take place in soil containing 1,500 lb of nitrogen as NaNO_3 or 1000-2000 lb. of nitrogen as clover tops per acre. The presence of total nitrogen in the soil up to 3000 lb. per acre does not affect nitrogen fixation by cowpea. Five plants of cowpea in a pot have been found to fix 1295 mg. of nitrogen.

By application of nitrogenous fertilizers, the normally formed legume nodules are rendered entirely inactive [Beidermans, 1918]. The amount of nitrogen fixed is inversely proportional to the amounts of soil nitrogen available to the plant; the effect of nitrate is similar. In early stages nitrogen fixation takes place best when small amounts of nitrogen are supplied to the plant till the flowering stage [Giobel, 1926]. Application of mineral nitrogen

(3 mg. per plant) to inoculated peas kept at low temperatures had very little effect on the amount of dry matter produced. Nitrogenous fertilizers lower the amounts and percentage of total nitrogen present as protein and amino-nitrogen in crops, but the nitrate application somewhat lowered the nitrogen content of the crop [Vaitiovaara, 1937]. Thornton [1936, 2] and Thornton and Nicol [1934] have shown that the application of mineral nitrogen does not damage a leguminous crop when grown by itself; the legumes obtain their nitrogen from these compounds instead of through activity of the nodules. However, they adversely affect the growth when the fertilizers are applied to a mixed crop of legumes and non-legumes.

Walker and Brown [1935] found that, in general, the application of manure, limestone and phosphate fertilizers to soils served to increase to a large extent the numbers of both the alfalfa and red clover root-nodule bacteria.

It is generally recognized that the leguminous plants are of great economic importance in agriculture. The benefit which they confer on the soil is principally due to the nitrogen compounds elaborated in the root nodules and subsequently released in the soil [Stallings, 1926]. The amounts of nitrogen fixed by various leguminous crops under field conditions have been estimated by several workers and have been found to average to about 100 lb. per acre annually. Analysis of soil under clover, carried by Shutt [1931] over a period of ten years, showed that this crop enriched the soil in nitrogen at an average rate of 50 lb. per acre annually. The amount of nitrogen added to the soil depends on the nature of the soil and the amount of nitrogen available in the soil. The poorer the soil the larger the amount of nitrogen taken from the air. Whiting [1915] has suggested that about two-thirds of the nitrogen of legumes grown on soils of normal productive power is obtained from the atmosphere. On this basis he has estimated that a 3-ton crop of cowpea hay takes 86 lb. of nitrogen, a 25-bushel crop of soybean 106 lb., a 4-ton clover 106 lb., and a 4-ton alfalfa 132 lb. The soil enrichment thus produced by legumes may last for several years [Nicol, 1933]. Harrison [1915] has described the preparation of nitro-cultures and their commercial application.

(iii) *Higher forms of life*.—Among the higher forms of life that have the power of fixing atmospheric nitrogen the algae seem to be of some importance. The more recent researches into the fixation of nitrogen by algae have shown that these play an important part in the fixation of nitrogen in the water-logged soils, especially in the rice fields of India.

Inoculation experiments

A good deal of work has been done for increasing the soil nitrogen content by inoculating the soil with suitable organisms. The various attempts made during recent years in this direction may be briefly summarized as follows.

(i) *Azotobacter*.—Emerson [1918] has shown that although inoculation of soil with *Azotobacter* is possible and practicable, it has no effect on the amounts of non-protein, amino or polypeptide nitrogen in the soil and there was no accumulation of these forms of nitrogen in soil under field conditions. Lipman [1908] and Lipman and Brown [1907] have reported that inoculation with *Azotobacter* in presence or absence of organic matter decreased rather than increased the yield, dry matter and nitrogen content of corn crops. Bottomley [1910] has, however, obtained increased yield in pot experiments.

with barley, *Avana sativa*, galtonia and parsnips by inoculating seeds with mixed culture of *Azotobacter chroococcum* and *Pseudomonas radicicola*. He [1914] has also shown that when specially treated peat containing nitrogen-fixing organisms are added to soils in pots, there is increase in total nitrogen of the soil resulting in large increased growth of a variety of plants. Stoklasa [1909] has obtained better yields of oats, potatoes and beets by soil inoculation in field experiments. Brown and Hart [1925] have found that wheat yield was not increased although nitrogen accumulated in the soil as a result of inoculation with *Azotobacter*. Inoculation with 'nitrofer' has been found to be effective in increasing nitrogen fixation in the soil [Zucker, 1928]. Makrinoff [1929] has reported good results from inoculation with non-symbiotic bacteria. More recently, Karunakar and Rajagopal [1937] have obtained significant increase in yield of grain and straw in sorghum by inoculation of seeds with *Azotobacter*; there was greater response by the addition of CaCO_3 and K_2HPO_4 . Martin and Brown [1937] have, however, found that inoculating with *Azotobacter* increased the dry weight and the total nitrogen in timothy grass but not in corn and wheat.

For successful inoculation suitable carbohydrate, sufficient air supply, lime, P_2O_5 , K_2O and other essential mineral nutrients in the soil are necessary [Stoklasa, 1909; Vandecaveye and Anderson, 1934; Omeliansky, 1915]. Kreybig [1929] has emphasized the importance of soil reaction for successful inoculation. There was greater bacterial activity in inoculated lime plots [Martin and Brown, 1937]. Eugel [1931] has shown that *Azotobacter* nitrogen (dead or alive) is easily nitrified in the soil.

(ii) *Legume bacteria*.—Ball [1907] has found from pot experiments that in artificial inoculation of 'nitroculture' the number and vigour of the tubercles were not as great as that occurring by natural means. Inoculation of seed with nitroculture leads to increase in nitrogen; potassium and phosphorus fertilizers gave a further increase [Wright, 1908]. Soil and seed inoculation with nitragin and nitrobacterine increased yield in lupines and sand peas, especially in the presence of phosphate. The nitrobacterine showed a greater effect than nitragin in soil poor in lime [Grabner, 1910]. Inoculation of undecomposed virgin Shapgnum moor soil with nitragin and azotogin are found to have very good action on yellow lupines; farmogen was almost inactive [Ferlitzén and Nystrom, 1914]. The soil conditions for the application of nitragin are deficiency of nitrogen, soil reaction and presence of sufficient amounts of other fertilizing ingredients and pH [Anon, 1919]. Growth of lucerne in south-east Scotland soil took place between pH 6 and 7; and no growth was observed between pH 5.0 and 5.49. Inoculation with bacteria resulted in an increase of 100 per cent total nitrogen of dry matter. Some strains were more effective than others [Cunningham, 1928]. Nolte [1919] has reported that three of the bacterial nitrogen fertilizers that he tried are found to be of limited value as sources of supplying nitrogen to the soil.

Brown and Stahlings [1921] have found from pot experiments using inoculated clover and alfalfa that when the hay crops are removed, there may be some gain in nitrogen in the soil. Inoculation of the nodule bacteria of lucerne increased the nitrogen content and produced increased crop yield [Razwumovskya, 1934]. Practical legume inoculants containing two or more

legume cultures compare favourably with standard group single cultures with respect to efficiency of nitrogen fixation and nodule formation [Bond, 1938].

Light, heat and exposure do not affect soil cultures as much as agar or liquid cultures [Fellers, 1919]. Drought and heat have only a temporary effect on legume bacteria in Poulouse silt loam [Vandecaveye, 1925]. The poor development of nodules in acid soil is due to the effect of acidity on the bacteria during the interval they exist non-symbiotically [Karrakar, 1927]. Vandecaveye [1927] has shown that *Rh. leguminosarum* is capable of surviving wide extremes of moisture and long periods of absence of host plant; it is distributed by wind and dust storm to a slight extent. Soybean nodule bacteria is relatively short lived, rarely surviving a year [Wilson, 1934]. Movement of *Astragalus sinicus* (genge) is largely influenced by moisture content (optimum being 18 per cent and no movement at less than 5 per cent) and soil concentration. There is strong chemotactic action between bacteria and genge seeds [Itano and Matsuura; 1934]. Hofer [1938] has shown that five to forty bacterial cells are necessary for successful inoculation of clover and alfalfa seeds. Asparaginate in place of yeast extract is useful in the medium for distribution of cultures. For best performance, the culture for inoculation should carry at least 80 millions of bacteria per lb. of seeds.

Joshi [1920] has found that the root nodule bacteria exert a beneficial influence on graminaceous plants also. By experiments with porous cylinders he has shown that soluble products are excreted into the soil. Stahlings [1926] has shown that wheat grown with inoculated soybeans may under favourable conditions obtain considerable amounts of nitrogen from the latter, with a lowering in their nitrogen content. It contained a higher percentage of nitrogen than wheat grown alone. Decrease in acidity of soils leads to increase in nitrogen content in leguminous plants; at pH 6 it is 30 per cent higher than at pH 5. Demelon and Dumez [1938] have shown that the same soil fatigue phenomena due to continued legume culture occurring with clover, lupine, peas, beans and soybeans is the same.

A thick close crop in crimson clover favours an early accumulation of nitrogen, the first month of growth yielding one third the total. The distribution of nitrogen in different parts of the plant varies greatly, about a third on an average being in the root [Penny and Macdonald, 1909]. Smith [1912] has reported that the number of *Rhizobia* present varies from 3—4 millions per gram of soil; the number of the organisms affords an index of the fertility of the soil. Application of nitrogenous manure during the early period of growth of inoculated plants produce good results by preventing any injury during the period of hunger [Ritter, 1911].

(iii) *Seed, root and plant inoculation.*—Dunham and Baldwin [1931] have indicated the necessity of using only effective strains of the nodule organism for seed inoculation since definite detrimental results may occur by the use of ineffective strains. Nobbe *et al.* [1909] have found that pure culture of bacteria obtained from a kind of legume works symbiotically with other species of the same genus of plants. There are, however, genera of legumes, such as pea and vetch, serradella and lupine, in which reciprocal inoculation increases the supply of nitrogen in the soil.

Inoculated seeds should not be stored for long periods before sowing, but the delay of several days or even a month may not do great harm [Fellers, 1918].

From a comparative study of the nitrogen content of seeds and inoculated plants Whiting and Schooner [1920] have shown that marked fixation of nitrogen takes place after the formation of the first leaf, 19 days after planting. The first appearance of nitrogen fixation was nine days after planting and by 26 days the amount of nitrogen fixed was three times that contained in the original seed. The older seeds of a given legume are more acid than the fresh seeds [Wilson, 1939, 1]. Thornton [1929] has shown that the appearance of nodules coincides with the opening of the first true leaf. The active substance inducing nodulation is not formed by the leaf, for the removal of leaf while still closed has no influence on nodule appearance. Plants containing sufficient nitrogen are immune to further infection of radiclecola [Lohnis, 1930]. Link [1937] has shown that B-indolacetic acid is one of the agents, if not the agent, responsible for the incitation of nodulation in susceptible hosts. The effect of such hetero-auxones may account for the beneficial effect obtained by green manuring with nodule forming plants and by manures, composts and humus soils. Chemicals have very little effect on nodulation. Treated seeds produce larger nodules than the untreated [Kadow *et al.*, 1937].

Mixed culture studies

So far, few attempts have been made to study nitrogen fixation by the mixed flora of the soil which is the nearest approach to soil conditions. The precise manner in which the nitrogen-fixing organisms, especially *Azotobacter* and *Clostridium* function in the soil where they have to compete with the other soil organisms and the extent to which the combined activities of all these organisms contribute towards nitrogen fixation in the soil are not well understood. The recent investigations of Bhaskaran and Subrahmanyam [1937] with the mixed flora of the soil have shown that the study of nitrogen-fixing organisms in pure cultures are only of limited value in explaining the mechanism of the process occurring in the soil. They have reported that the fixation of nitrogen by the mixed flora of the soil follows a different course from that of a pure culture of *Azotobacter* alone in artificial media. The latter is comparatively slow in decomposing sugar, and the fixation proceeds only so long as the sugar lasts in the medium. The residual matter is not utilized to an appreciable extent in the fixation of nitrogen. On the other hand the mixed flora of the soil though fewer in number rapidly decompose the sugar. Only a small quantity of nitrogen is fixed in presence of sugar while the major part amounting to over two-thirds of the total quantity fixed, is fixed in the later stages [Bhaskaran and Subrahmanyam, 1936]. They [1937] have further shown that the products of decomposition of sugar are utilized in this subsequent fixation.

The above observations would suggest that although *Azotobacter* may be potent by itself in the early stages of sugar decomposition, it does not play a large part in nitrogen fixation in presence of other organisms of the soil. The latter decompose the sugar at a rapid rate, so that it will receive only a limited amount of the organic nutrient and will, in consequence, fix only a small amount of nitrogen. The fixation that takes place in the soil after the disappearance of sugar is presumably due to the other organisms present in the soil.

Bhaskaran and Subrahmanyam [1937] have also shown that the residue left after the decomposition of sugar is highly potent in fixing nitrogen in the soil. Thus, there is threefold increase in the amount of nitrogen fixed by the mixed flora of the soil when the residue is used as energy material in place of sugar. From the agriculturists' point of view, this observation is of considerable practical importance.

DISCUSSION

From the evidence so far collected on the question of fixation of atmospheric nitrogen by living forms, it would appear that the capacity of fixing nitrogen is mainly confined to unicellular organisms. The distribution of nitrogen-fixing power is not, however, manifested in any one stage of evolution of life. Perhaps the algae are the most primitive organisms which have the power of fixing nitrogen. Probably the processes of carbon and nitrogen assimilation, as manifested in the blue green algae *Anabena*, are coeval in the process of evolution and this must have been the case in order that any living organism could have ever appeared on this earth. The fact that organisms living under entirely different environmental conditions, such as, in the absence of molecular oxygen, in presence of plenty of oxygen and in the living tissues of higher plants fix nitrogen, would show that the function of nitrogen fixation was more universal among the living organisms once upon a time. Later on, with evolution only a few of them retained the power so as to maintain the stock of combined nitrogen in the soil.

The process of fixing elementary nitrogen is not, however, identified with the life of the organism. The organism does not fix nitrogen in presence of readily available combined nitrogen in the medium. Further, they do not depend on the nitrogen-fixing process (unlike the autotrophic bacteria which depend on nitrification) for their maintenance. The process of nitrogen fixation being a secondary life process, it is probable that the fundamental mechanism involved in nitrogen fixation is similar in the different forms. The study of the chemical changes that are involved in nitrogen fixation apart from the general metabolism of the organisms has been of great scientific interest.

The nitrogen-fixing reaction requires a source of energy, the presence of minerals Ca (replaceable by Sr) and Mo (replaceable by V) and an optimum reaction. In view of the uncertainty of the first chemical step in nitrogen fixation it is not known whether the process is exothermic or endothermic. Whether it fixes nitrogen or not the organism uses the same amount of energy material (carbohydrate) for its growth. These observations would suggest that the energy material has no part to play in the chemical mechanism of nitrogen fixation. It is, however, difficult to understand how small concentrations of Ca and Mo (replaceable by Sr and V respectively) have a specific role in nitrogen fixation.

The nitrogen-fixing reaction in the living forms is essentially a chemical process. The changes of the N_2 molecule to form part of the bacterial protein are so rapid that it has not been possible to know the different stages of the reaction. To get a complete picture of the scheme of reaction, it is necessary to isolate the nitrogen fixing apparatus apart from the living cell so that the reaction could be arrested at the different stages and studied.

A consideration of the physico chemical data in regard to nitrogen fixation would show that the catalyst responsible in bringing about the reaction is an enzyme. The extra-cellular isolation of the enzyme has not, however, been possible. The future line of work therefore consists in the isolation of this enzyme and study of its exact chemical nature with a view to finding out the active chemical group in it which is ultimately responsible for the nitrogen-fixation reaction.

In addition to its theoretical interest the problem of biological nitrogen fixation is of considerable practical importance. So far, the various attempts to use the nitrogen-fixing organisms as a source of supplying nitrogen to the soil for increased plant growth has not been successful. Probably a fuller understanding of the chemical mechanism of nitrogen fixation would enable the farmer to make use of this biotic energy for increased crop production.

SUMMARY

1. The various forms of life that fix atmospheric nitrogen in nature have been classified into (a) the non-symbiotic or free living bacteria, (b) the symbiotic or legume bacteria and (c) higher forms of life consisting of algae, fungi, plants, actinomycetes, germinating seeds of legumes and different vegetative parts of certain higher plants.

2. *Azotobacter* and *Clostridia* are the important non-symbiotic organisms which fix nitrogen in the soil. The *Azotobacter* is typical of the aerobes and the *Clostridia* of anaerobes.

3. *Azotobacter* is generally present in soils having pH above 6.0. The cells contain volutin bodies, fat and metachromatic granules.

4. The nutritional requirements of *Azotobacter* consist of a source of energy, water and certain minerals. The organism uses carbohydrates, salts of organic acids and alcohols as energy source. Soil humus has been found to exert a stimulatory influence on the organism for nitrogen fixation. Vitamin B₁₂ and phytonucleic acid stimulate growth and nitrogen fixation. Certain minerals in optimum concentration are necessary for the growth of *Azotobacter*, and among these calcium (replaceable by strontium) and molybdenum (replaceable by vanadium) are specific for nitrogen fixation; manganese and cerium compounds accelerate nitrogen fixation. Iron plays no specific role in the mechanism of nitrogen fixation.

5. *Azotobacter* respire at an enormously high rate. Spectroscopic examination of the cells reveals a respiratory mechanism similar to those ascribed to aerobic cells: the respiratory enzyme band is in the red region at 632 μ .

6. The activity of *Azotobacter* is dependent on the reaction of the media, temperature and air supply. The organism grows between pH 6.0 and 9.6. It grows and fixes nitrogen between temperatures 10 and 50°C., the optimum being between 34 and 35°C. Aeration of the medium facilitates nitrogen fixation.

There is, however, a marked influence on the amount of nitrogen fixed when the organism is exposed to light of different colours; yellow light is better than blue. It fixes more nitrogen in presence of protozoa, amoeba and certain species of bacteria and algae.

7. The organism does not fix nitrogen in presence of rapidly available combined nitrogen, 0.5 mg. per 100 c. c. media completely inhibits nitrogen fixation.

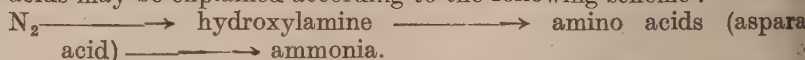
8. In dextrose media, *Azotobacter* produces formic, acetic, lactic and tartaric acids and ethyl alcohol; a large part of the sugar is converted into carbon dioxide.

9. The cells consist chiefly of carbohydrates, proteins and a small percentage of minerals. The proteins are very similar to those of other organisms except for a high content of arginine.

10. In culture media *Azotobacter* produces a characteristic slime. It is a carbohydrate, and is levo-rotatory and it belongs to the class of true gums.

11. With ageing in culture medium the organism produces characteristic pigments; the pigment is a melanin of unknown nature formed from tyrosine by the action of tyrosinase.

12. Nitrate, ammonia, hydroxylamine and amino acids have been reported by different workers as the first intermediate product in the fixation of nitrogen by *Azotobacter*. Direct experimental evidence for the oxidation of nitrogen to nitrate is lacking. Detection of ammonia, hydroxylamine and amino acids may be explained according to the following scheme:—



It is therefore likely that the first intermediate product is hydroxylamine and that the amino acids and ammonia detected represent the later stages of the reaction.

13. The properties and behaviour of the nitrogen-fixing system in *Azotobacter* are, however, characteristic of an enzyme reaction. It has been considered as a phycenzyme and named azotase. The specific component within the azotase system which combines with the N_2 molecule is termed nitrogenase. The auxiliary substances known at present for the enzyme reaction are calcium (replaceable by strontium) and molybdenum (replaceable by vanadium) and hydroxyl ion. The extra-cellular isolation of azotase and the study of the enzyme apart from the growth and general metabolism of the organism has not so far been possible.

14. *Clostridia* are present in almost all soils of the world and are found in rather large numbers in acid soils. They occur more abundantly than *Azotobacter*.

15. The different species of *Clostridia* have not been clearly defined. *Clostridium pasteurianum* is typical of the species fixing nitrogen, and it is an obligate anaerobe.

16. The organism can be easily isolated from soil by using Winogradsky nutrient medium and by prolonged pasteurization at 75°C . The optimum temperature for the development of *Clostridium pasteurianum* is between 25°C and 30°C . and the optimum reaction is between pH 6.9 and 7.3.

In presence of oxygen the organism forms characteristic spores. The spores are not destroyed at 75°C . even at the end of 15 hours. They could be preserved in the dry state for 20 years with the nitrogen-fixing power intact.

17. In sugar media characteristic butyric fermentation takes place;—45 per cent of dextrose is converted into a mixture of acetic and butyric acids in varying proportions; small amounts of alcohol (ethyl, propyl and

tyl) are formed and a considerable evolution of a mixture of carbon dioxide and hydrogen also takes place.

18. In nitrogen-free media the organism fixes about 3 mg. nitrogen per gram of sugar decomposed. The greater the concentration of sugar, the lower is the economic utilization; 3.2 mg. nitrogen is fixed in 0.5 per cent glucose solution, 2.0 mg. in 2 per cent solution and 1.2 mg. in 4 per cent solution.

Combined nitrogen in the medium reduces nitrogen fixation; presence of 0.1 per cent of combined nitrogen in 1,000 parts of medium has been found to completely inhibit nitrogen fixation.

19. It is claimed that a large number of free-living bacteria present in soil other than *Clostridium* and *Azotobacter* have the power of fixing nitrogen. But it is doubtful whether these are of any importance in soil nitrogen fixation.

20. A group of soil bacteria known as *Rhizobia* has been found to infect the roots of leguminous plants and develop characteristic nodules; the bacteria, in association with the plant, fix atmospheric nitrogen.

Six species have been recognized in this group of bacteria, viz. *Rh. leguminosarum* Frank, *Rh. trifolii*, *Rh. phaseoli*, *Rh. meliloti*, *Rh. japonicum* and *Rh. ciceri*.

21. Whether grown in culture media or soil, the bacteria exhibit a clear definite life cycle which consists of five stages: (a) the small non-motile swarmer coccus, (b) the larger non-motile coccus, (c) motile swarmer, (d) rod form and (e) the stage of high vacuolation (bacteriod).

This distinct life-cycle has a bearing on the spread of bacteria through soil and consequently on the infection of the host plant. Special reproductive cells, gonidia or spores are not formed in the process of reproduction.

22. The nodule bacteria can be easily cultivated in artificial media. The optimum pH for the growth of the nodule bacteria in nutrient gelatin lies between 6.5 and 7.5. The bacteria present in different leguminous plants are divisible into two physiological groups according to their cultural characteristics and biochemical reactions. The organisms present in alfalfa, clover, pea and dahlia produce an acid reaction in sugar media while those of bean, cowpea and lupine an alkaline reaction.

23. The appearance of the nodule on the seedling takes place with the unfolding of the first true leaf; the removal of the leaf, however, does not prevent nodule formation. The active substance secreted by the bacteria produces a weakening of the cell-wall and bacteria enter the root at this point.

There is a marked specificity in the infection of host plant by bacteria; only a few host specific species have so far been recognized. Infection of the host plant outside the specific group is of rare occurrence.

24. The entry of the bacteria into the root hair produces important histological changes in the host tissue leading to rapid division of the root cells and formation of nodular tissue. The bacteria are distributed through the nodules in three different ways in different legumes: entry through perforations in the cell wall, infection of the inter-cellular spaces and invasion of the meristematic cells. The cytoplasm of the infected cells becomes closely packed with bacteria which later on become branched and constitute the so-called 'bacteriods'. A group of these bacteriods form the nodule.

25. Neither the bacteria nor the host plants can fix nitrogen by themselves. The process of nitrogen fixation is therefore the result of symbiotic relationship between the plant and the bacteria. The nitrogen fixed in nodular tissue is translocated to the different parts of the plant.

26. The proper functioning of the bacteria within the host depends upon the maintenance of a nice physiological equilibrium between the host and the bacteria. Presence of nitrate, ageing of the nodules and food supply may disturb this equilibrium. An adequate and unhindered carbohydrate supply is essential for the healthy functioning of the nodule.

27. Natural humic acid and iron salts stimulate growth of *Rhizobium*. Adsorbed calcium is useful in transforming the abnormal nodules into effective nodules. Titanium salts have specific morphological influence on the bacteria. The supply of exchangeable bases is a limiting factor in legume growth and nitrogen fixation.

28. Maximum growth and respiration take place near neutrality or at a constant activity between pH 6.0 and 7.8. The effect of soil acidity on nodule formation is not due to its effect on the growth of roots of the plant but due to its effect on bacteria when it exists in the soil non-symbiotically.

29. Yeast extract, molasses, sauerkraut and extracts of leguminous plants contain accessory growth substances which greatly influence the growth of nodule bacteria. They provide an initial H donor which in turn lowers the rH and supplies a readily available initial source of energy.

These extracts contain a secondary accessory factor which in conjunction with vitamin B₁ is highly active in promoting the growth of bacteria. This effect is due at least in part to the presence of thiamin and flavin in those extracts.

The beneficial effect of certain extracts may be due to their effect on the oxidation-reduction potential of the medium.

30. The formation of bacterioids in the nodosities is also dependent upon the presence of alkaloids in the roots. Caffeine, quinine and strychnine in small doses stimulate growth of bacteria in liquid cultures.

31. The bacteriophage isolated from nodes of leguminous roots is active in dissolving the bacteria; the lytic action is specific.

32. The presence of rapidly available combined nitrogen in the soil increases nitrogen fixation in the nodules; the influence depends on how it affects the C : N relationship in plants.

Small amounts of amino acids result in the loss of infective ability of *Rhizobia*.

33. With nitrogen fixation the organism produces a characteristic slime. It is produced in faintly alkaline and neutral media but not in acid media. It is a polysaccharide containing glucuronic acid.

34. The composition of the nodular tissue is not different from that of other parts of the plant. The proteins are similar to those of *Azotobacter*. 20 per cent of the nitrogen of the nodule is arginine.

35. In sugar media the bacteria produce acids, alcohol and trace amounts of aldehyde; the fermentation is of the pyruvic acid type.

36. Evidence has been adduced to show that in symbiotic nitrogen fixation the nitrogen molecule is first converted into hydroxylamine through an unknown intermediate. The hydroxylamine reacts with the oxalacetic

the plant to form oxime which is then reduced to give *l*-aspartic acid. The evidence in support of this scheme of reaction is, however, inadequate.

37. The nitrogen compounds excreted by nodules are mainly amino acids; aspartic acid accounts for 50 per cent of the amino acids, while the other half probably is lysine. The excretion takes place right from the bacteria inside the nodules and not from the roots. Direct contact of the roots with nodules with solid particles is necessary for nitrogen excretion. The excreted nitrogen represents the primary product of nitrogen fixation and not a decomposition product of the proteins.

38. The rate of respiration of nodule bacteria is considerably higher than that of other bacteria. The respiratory quotient (*R. Q.*) is higher in *Rh. clostrii* than in the other species. The rate of oxygen consumption increases with increased oxygen pressure in the atmosphere.

Yeast extract, cane molasses, humic acid and commercial egg albumin increase the rate and extent of oxygen consumption by all strains of *Rhizobia*. This may be due to the presence of a coenzyme in those substances, which is essential for respiration.

39. Symbiotic nitrogen fixation has also been considered as an enzymic reaction. Nitrogen fixation is dependent on the pressure of nitrogen (pN_2); the thermodynamic dissociation constant (kN_2) is 0.05 ± 0.005 atmospheres, whereas in the non-symbiotic organism (*Azotobacter*), it is 0.215 ± 0.002 . The relation of the nitrogen-fixing reaction to oxygen pressure being independent of the source of nitrogen, it is probable that molecular oxygen is not directly concerned in the fixation process. In presence of H_2 , however, there is an intimate relationship between the enzyme system responsible for fixation of free nitrogen and the oxidative system present in it. Molecular hydrogen inhibits symbiotic nitrogen fixation whereas it is quite inert towards non-symbiotic nitrogen fixation brought about by *Azotobacter*.

40. Certain species of algae, yeast, fungi, germinating seeds of legumes and certain plant cells have been found to fix atmospheric nitrogen.

41. Three species of the blue green algae, *Anabaena* isolated from the rice fields of India have been found to fix atmospheric nitrogen.

42. The evidence for fixation of nitrogen by free living fungi, yeasts and mycorrhizal fungi is, however, inadequate. But mycorrhizal fungi in association with the roots of ericaceous plants utilize atmospheric nitrogen.

43. In view of the conflicting evidence it is difficult to draw any definite conclusion regarding the ability of germinating seeds of legumes to assimilate atmospheric nitrogen.

44. It is claimed that roots and leaves of certain non-leguminous plants inhibit the power of fixing atmospheric nitrogen either by themselves or by their association with the bacteria present in them.

45. The nitrogen-fixing bacteria in the soil are the chief agents responsible for maintaining the store of soil nitrogen.

46. The relative importance of the various non-symbiotic bacteria responsible for the increase of soil nitrogen is not well understood. *Azotobacter* is present in almost all soils of the world and is more potent in tropical soils. Our knowledge of *Clostridium* in its relation to soil fertility is, however, meagre; probably this fixes nitrogen anaerobically in the deeper layers of soil.

47. The leguminous plants are of great economic importance in agriculture. The nitrogen fixed in their nodules is released in the soil for plant nutrition. The soil enrichment thus produced by legumes may last for several years.

48. Artificial inoculation of *azotobacter* and other non-symbiotic nitrogen fixing organisms in the soil has not so far proved successful in general agricultural practice.

49. The various attempts at soil, seed and plant inoculation with commercial cultures of legume bacteria are described. It is possible by soil inoculation to improve the growth of legumes in regions where non-effective strains of nodule bacteria predominate in the soil.

50. The fixation of nitrogen by the mixed flora of the soil follows a different course from that of pure cultures of the nitrogen-fixing organisms on artificial media. The economy of carbon utilization in nitrogen fixation by the mixed flora of the soil is different from that of *Azotobacter* in pure culture.

When the products of anaerobic decomposition of sugar are used in place of sugar as energy material, there is threefold increase in the amount of nitrogen fixed by the mixed flora of the soil.

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ADDENDUM

Since this paper was communicated for publication, a large volume of work has been done, particularly on the biochemical aspects of nitrogen fixation. A brief reference may be made to the more important of these researches.

Azotobacter

Bortels [1939; 1940] and Burk and Horner [1940] have further shown the need of molybdenum in growth by *Azotobacter*. At the same time it is becoming increasingly clear that although all nitrogen-fixing organisms so far tested require molybdenum (or vanadium), iron, and calcium (or strontium), in no case can it now be considered as probable that these elements are specifically required in the nitrogen-fixation process as distinguished from general assimilation of combined nitrogen. The only qualitative fixation specificity that can be regarded as established at present is hydrogen inhibition, and even this is probably essentially physical rather than chemical.

Burk [1941] has shown that growth of *Azotobacter*, in both free and fixed nitrogen requires, as in the case of most if not all other bacteria and higher forms, a minimum concentration of carbon dioxide; inhibition of *Azotobacter* growth by too low pressures of carbon dioxide (0.05 per cent or less) is readily observed in a Warburg apparatus by maintaining too effective absorption of respiration carbon dioxide in the alkali, with very dilute cultures. Carbon dioxide about 1 per cent or more lowers (reversibly) the concentration range over which nitrite is toxic for respiration and growth.

Wyss and Wilson [1941] have reported that essentially all the findings on inhibition of symbiotic fixation by hydrogen [Wilson, 1940] hold also for fixation by *Azotobacter*. Burk and Burris [1941] have confirmed these observations, which obviously introduce a new unity into our conception of the processes of fixation in *Azotobacter* and in legume root nodules. Acceptance of these new findings necessitates a re-interpretation of the hyperbolic function obtained in the experiments of Lineweaver, Burk and Deming several years ago.

Horner and Burk [1939] have observed that young cultures of *Azotobacter* vigorously fixing nitrogen generally excrete some 10-25 per cent of the nitrogen into the surrounding medium. The extra-cellular nitrogen is quite a heterogeneous mixture: about two-thirds precipitable by lead acetate, one-third by phosphotungstic acid, one-fifth by aluminium phosphate and still less by trichloroacetic acid. Winogradsky [1939, 1, 2], in support of his previous observations, has adduced evidence that autolyzing silica gel cultures of *Azotobacter* yielded more ammonia nitrogen after disappearance of the organic substrate than corresponded to the simultaneous loss of total nitrogen. In his opinion, a highly efficient enzymic synthesis of ammonia was involved, which accumulated slowly over a period of months a relatively important amount of ammonia, under conditions that might well obtain in soils or waters. The evidence is still not convincing.

Clostridium

The present authors have obtained evidence that during decomposition of glucose by *Clostridium pasteurianum*, there is very little fixation of nitrogen in the first 10-12 days, although during this period there is rapid conversion of the sugar carbon into volatile acids and other soluble products. The major part of the fixation takes place only after the sugar has disappeared from the medium. During this period there is little loss of carbon from the medium, the efficiency of nitrogen fixation being of the order of 1:19. There is greater intake of carbon than nitrogen by the cells during the early stages of growth. The nitrogen thus fixed is mostly present in the residue consisting of bacterial cells.

It may, therefore, be concluded that nitrogen fixation by the mixed flora of the soil in sugar media takes place in two stages: the first stage of aerobic fixation which continues until the added sugar disappears and in which *Azotobacter* is the chief organism concerned in the fixation; this is followed by the second stage in which *Clostridium* contributes to the nitrogen fixation. From the point of view of carbon utilization, the efficiency of nitrogen fixation in the later stage is much more favourable than in the first stage. It would appear that *Clostridium* is of greater importance than *Azotobacter* in soil nitrogen fixation.

Rhizobium

In his monograph Wilson [1940] has dealt with the different aspects of symbiotic nitrogen fixation (chiefly biochemical) that have undergone considerable development in the last decade—the biochemistry of the bacteria in pure culture, the interaction of host plant and bacteria, the chemical mechanism of the fixation process, the effect of the carbohydrate-nitrogen balance in the host on fixation, excretion of nitrogenous substances from nodules into the rooting medium, physico-chemical studies of possible enzyme systems, and a discussion of practical applications.

Virtanen [1939] has claimed that legumes utilize aspartic acid in preference to all other forms of fixed nitrogen, and that non-legumes (wheat, barley) use it scarcely at all, a claim of great significance for the hydroxylamine (oxime) theory of legume nitrogen nutrition. The weakness of this theory is that neither hydroxylamine nor oxime has ever been clearly shown to be an available source of nitrogen for nutrition of either symbionts or *Azotobacter* even at non-toxic concentrations.

Virtanen and Laine [1939], and Virtanen and Törnainen [1940] have further shown that under the widely varied and studied experimental conditions used by them leguminous plants almost invariably excrete into the soil considerable amounts of the nitrogen fixed from the atmosphere. The amounts excreted are greater than can be accounted for by sloughing-off of nodules, and excretion frequently occurs early in the life of the plant before appreciable decay or sloughing-off would be anticipated. The objection that non-symbiotic nitrogen fixation is responsible for the observed effect is answered by experiments carried out by Virtanen and his co-workers under bacteriologically controlled conditions. Wilson and Wyss [1939], Bond and Boyes [1939], Romashev [1939], and

Ludwig and Allison [1940] have obtained negative results. Slight, but questionable excretion has been observed by Shapter [1939] and by Madhok [1940]. Variable results with some positive findings have been recorded by Scholz [1939]. Wilson [1940] concludes that excretion is obtained only under particular conditions, namely, those providing sufficient photosynthesis to ensure a fairly high rate of nitrogen fixation but without excess of carbohydrate to bind into the plant all nitrogen as it is fixed.

According to Kubo [1939] the 'red body' present in nodules, which Pietz describes as an oxidation product of dihydroxyphenylalanine, is a hemoprotein. He found the hemoprotein, which he has isolated from the nodules of many leguminous species, dissociation, gives a hemin identical in crystal form with the hemin from horse hemoglobin. He reported that the hemoprotein stimulated succinate oxidation by *R. japonica*. Link and Eggers [1940] found that nodules of beans and peas had different auxones and a higher auxone content than roots grown on sterile substrates. Georgi and Beggs [1939], however, found that the soil organism *B. radiobacter* produced auxin at a much rapid rate than the root nodule bacteria. Evidence that auxins are really critical in the formation of the highly differentiated tissue of root nodules is as yet merely suggestive.

In their initial publication Allison, Hoover and Burk a few years ago observed that coenzyme R is not identical with the complex, bios, and left open the question of its relationship to components thereof. Nilsson *et al.* [1939, 1, 2], and West and Wilson [1939, 1940] showed its virtual identity with bios IIB, or biotin. Still further confirmation was later provided by Gyorgy *et al.* [1940, 1], who concluded that these two substances were also identical with vitamin H, a conclusion that was further established by Vignier *et al.* [1940] and Gyorgy *et al.* [1940, 2]. The establishment of the identity of biotin, coenzyme R, and vitamin H is obviously of great significance for connecting plant and animal vital economy, the more so in view of the already demonstrated rôle of coenzyme R in respiration and hence probably in fundamental intermediate metabolism, and also in view of the many directions which research on biotin has taken in 1940, since its connection with animal metabolism has become known.

So far, no direct rôle of coenzyme R is indicated in the nitrogen-fixation process. This possibility is not to be excluded, however, in view of the fact that the effect of coenzyme R in *Rhizobium* respiration involves the presence of readily available nitrogen [Allison and Hoover, 1939], as has also been demonstrated by Burk *et al.* [1941] in effect on both respiration and fermentation of yeast.

Ruben *et al.* [1940] have made an isotopic approach to the study of nitrogen fixation. They exposed tops of barley plants to purified radioactive nitrogen, N^{15} , for 20 minutes and then subjected the tops to a hot alcohol extraction; the extract was next boiled in a stream of air. An extract from a control plant, killed by boiling water, showed no radioactivity, whereas the extract from the live plant contained small amounts of N^{15} . As the authors stated, the evidence indicates fixation of nitrogen, but the possibility of exchange has not been eliminated, and more details and control experiments are needed for the data to be convincing.

Algae

Referring to the recent work of De [1939] suggesting that certain algae, particularly those from some rice fields in Bengal (India), are able to fix atmospheric nitrogen, Chaudhuri [1940] has reported that his own work leads to the view that bacteria (*Azotobacter*), living in the mucus sheath of these algae, are responsible for nitrogen fixation.

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STUDIES WITH WHEAT UNIFORMITY TRIAL DATA

I. SIZE AND SHAPE OF EXPERIMENTAL PLOTS AND THE RELATIVE EFFICIENCY OF DIFFERENT LAY-OUTS

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(With two text-figures)

THE conclusions of any field experiment are based mainly on the pooled estimate of the variance within the different replications after eliminating for treatment effects. This variance, as is well known, is influenced by a number of causes, the most important of them being the size and shape of plots, the design of the lay-out and the extent of heterogeneity present in the site selected for the experiment. Uniformity trials, as indicated by Cochran [1937], enable us to improve the methods of field experimentation by obtaining information on the three points mentioned above.

A review of the literature on uniformity trials will show that most of them deal with the optimum size and shape of plots. The number of replications required for a certain degree of accuracy has also been worked out in elaborate detail on the basis of these trials. The number of replications required for any experiment depends on the variation between plot to plot in the particular area selected for the experiment and also the degree of accuracy expected in the results. As these two factors are likely to change from field to field, the value of the findings from a particular uniformity trial regarding replications is not of any general use. But the trend shown by the studies on the plot size and the efficiency of different experimental designs is of immense value in improving the technique of field experiments.

The purpose of the present paper is to examine a wheat uniformity trial data with a view to obtain information on a number of points which will be of great use in laying out field experiments. The literature on the different aspects dealt with in this paper will be reviewed in brief when we discuss the respective results.

MATERIAL

The data of the present investigations were collected at the Agricultural Sub-station, Karnal, in April 1937, at the time of harvesting wheat I. P. 114 from the General Area No. 2 (Plot No. 38). The plot was sown on 11 November 1936 with a Monarch drill with 9-in. spacing between rows, and the crop was irrigated thrice before harvest. There was 0.74 in. of rain in May, 14.33 in. in June, 9.04 in. in July, 6.07 in. in August, 4.85 in. in September, 1.60 in. in December, 0.02 in. in January, 4.68 in. in February, 0.12 in. in March and 1.63 in. in April. The cropping, manuring

tural and other operations were uniform over the entire area. The previous cropping history was as follows :—

	<i>Kharif</i> (Monsoon)	<i>Rabi</i> (Winter)
1935-36	Fallow	Wheat I. P. 114
1936-37	Fallow	Wheat I. P. 114

A net area of 400 ft. \times 125 ft. (1.1478 acres) was harvested by dividing it into 80 \times 25 (or two thousand 5 ft. \times 5 ft.) units after eliminating a minimum border of 3.5 ft. all round the net area. All the plots were harvested and weighed individually. The outturn of every plot was recorded in ounces after thoroughly drying the grains in the sun. The yields are given in the appendix.

DISTRIBUTION OF PLOT YIELDS

Before taking the distribution of yields for the whole area, the homogeneity of the field was examined by dividing it into four parts or sets as indicated below :

- Portion included in set 1—1 to 25 rows and 1-20 columns
- Portion included in set 2—1 to 25 rows and 21-40 columns
- Portion included in set 3—1 to 25 rows and 41-60 columns
- Portion included in set 4—1 to 25 rows and 61-80 columns

The mean yield and its variance for the ultimate plot size 5 ft. \times 5 ft. are given in Table I for the four sets.

TABLE I
Mean and variance for the different sets

Sets	Mean (ounces)	Variance
1	17.198	8.808
2	15.498	7.875
3	16.066	8.675
4	17.818	9.570

Table I shows that the whole field cannot be considered as a homogeneous unit. However, there is justification to take sets 2 and 3 together, and sets 1 and 4 together.

The distribution of yields for a few plot sizes of sets 1, 2, 3 and 4 separately and together have been investigated by finding β_1 , β_2 , χ^2 , and $P(\chi^2)$ for normal distribution. Sets 1, 2, 3 and 4 have been taken together to see the changes in the values of β_1 and β_2 for varying plot sizes when the data are not homogeneous. Sets 2 and 3 are taken together to note the variations in β_1 and β_2 for different plot sizes when the data are homogeneous.

TABLE II

Distribution constants of yields for a few plot sizes

Description of plot size		β_1	β_2	χ^2	$P(\chi^2)$
Set 1.	5 ft. \times 5 ft.	0.186 ± 0.075	2.805 ± 0.202	44.4	0.01
	10 ft. \times 5 ft.	0.169 ± 0.084	2.461 ± 0.185	19.1	<0.05 and $>$
	10 ft. \times 10 ft.	0.061 ± 0.060	1.980 ± 0.149	13.9	>0.50 and $<$
Set 2.	5 ft. \times 5 ft.	0.144 ± 0.080	3.074 ± 0.295	23.5	>0.01 and $<$
	10 ft. \times 5 ft.	0.023 ± 0.020	2.580 ± 0.183	13.0	>0.20 and $<$
	10 ft. \times 10 ft.	0.197 ± 0.137	2.544 ± 0.301	22.6	>0.05 and $<$
Set 3.	5 ft. \times 5 ft.	0.300 ± 0.153	3.512 ± 0.558	22.1	>0.05 and $<$
	10 ft. \times 5 ft.	0.162 ± 0.105	2.859 ± 0.313	17.7	<0.05 and $>$
	10 ft. \times 10 ft.	0.099 ± 0.098	2.536 ± 0.272	28.4	<0.05 and $>$
Set 4.	5 ft. \times 5 ft.	0.057 ± 0.065	3.485 ± 0.519	31.0	0.01
	10 ft. \times 5 ft.	0.004 ± 0.006	3.339 ± 0.572	9.3	>0.5 and $<$
	10 ft. \times 10 ft.	0.000 ± 0.000	2.496 ± 0.225	11.1	>0.7 and $<$
Sets 2 & 3.	5 ft. \times 5 ft.	0.228 ± 0.087	3.361 ± 0.316	45.8	<0.01
	10 ft. \times 5 ft.	0.088 ± 0.051	2.806 ± 0.194	25.5	>0.01 and $<$
	10 ft. \times 10 ft.	0.156 ± 0.087	2.569 ± 0.211	35.6	<0.01
Sets 1, 2, 3 & 4	5 ft. \times 5 ft.	0.157 ± 0.045	3.163 ± 0.170	89.9	<0.01
	10 ft. \times 5 ft.	0.045 ± 0.016	2.763 ± 0.124	28.2	>0.01 and $<$
	10 ft. \times 10 ft.	0.031 ± 0.018	2.307 ± 0.096	42.9	<0.01

The distribution of yields for the three plot sizes 5 ft. \times 5 ft., 10 ft. \times 5 ft., 10 ft. \times 10 ft. of the sets 2, 3 and 4 can be considered to be normal for practical purposes. The values of β_1 and β_2 are not, on the whole, significantly different from 0 and 3 respectively. $P(\chi^2)$ also, more or less, confirms this conclusion. It may be noted that there is a tendency for the value of β_2 to diminish as the size of the plot increases. Set 1 cannot be considered to be normally distributed. Taking sets 2 and 3 together the distribution of yields for the smallest plot size is not normal. Values of β_1 and β_2 for the other sizes are not significantly different from those for the normal distribution, but the values of $P(\chi^2)$ show that the departure from normal curve, if not significant, is on the verge of significance. It is clear that the distribution for the whole area is not normal for any of the plots under consideration. Here also it is interesting to note that β_1 and β_2 diminish as the size of the plot increases. Briefly summarized the above investigations lead to the following conclusions:

- (i) The distribution of yields from smaller areas is more likely to be normal than from larger areas;
- (ii) There is a tendency for the value of β_1 to approach zero as the size of the plot increases;
- (iii) The value of β_2 though not significantly different from 3 in many cases, decreases as the plot size increases;
- (iv) The distribution of yields approaches normality as the plot size is increased.

OPTIMUM SIZE AND SHAPE OF PLOTS

We have already seen that the difference between the means for the separate plot sizes of sets 2 and 3 and also those of sets 1 and 4 are not significantly different from each other. This finding taken along with the values of β_1 and β_2 for these sets shows that the frequency distribution of the yield data for sets 2 and 3, and 1 and 4 could more or less be considered as belonging to the same universe. This was also confirmed by applying Pearson's [1932] χ^2 method of testing the probability of two samples belonging to the same universe. The values of χ^2 for sets 2 and 3, and 1 and 4 were 1.5 and 18.5 respectively with 13 degrees of freedom each. Hence investigations regarding the size and shape of plots were carried out separately for sets 2 and 3 together, 1 and 4 together and also for sets 1, 2, 3 and 4 together. The yields were computed for a large number of plot sizes, and the coefficient of variation for the different plot sizes, after eliminating for soil heterogeneity on the basis of blocks containing five plots running along rows as well as along columns, are given in Table III. This is also shown graphically in Figs. 1 and 2. In dealing with sets 1 and 4 together, care was taken to see that any block of five plots formed for purposes of eliminating soil heterogeneity did not extend from set 1 to set 4. The reduction in error by increasing plot size without any elimination for block effects will be seen from the coefficient of variation before elimination given in Table III. This will be useful in fixing the best size of plot for purposes of yield estimation by sampling before harvest.

TABLE III

Coefficient of variation for different plot sizes

Plot size (rows × columns)	Area in sq. ft.	Blocks running along rows							Blocks running along columns			
		C. V. before elimination			C. V. after elimination			Average of sets 1, 2, 3 & 4	C. V. after elimination			Average of sets 1, 2, 3 & 4
		Sets 1 & 4	Sets 2 & 3	Sets 1, 2, 3 & 4	Sets 1 & 4	Sets 2 & 3	Sets 1, 2, 3 & 4		Sets 1 & 4	Sets 2 & 3	Sets 1, 2, 3 & 4	
1 × 1	25	17.2	18.0	18.3	13.6	14.3	13.9	13.9	13.8	13.6	13.7	
1 × 2	50	14.7	15.7	16.0	12.0	12.5	12.2	11.8	11.2	11.3	11.3	
2 × 1		14.4	15.6	15.7	11.0	11.8	11.4		12.6	11.9	12.3	
1 × 3	75	14.0	14.4	15.2	11.2	11.3	11.2	10.8	10.5	10.3	10.4	
3 × 1		13.1	14.6	14.7	9.9	10.8	10.3		10.4	9.4	10.0	
4 × 1	100	12.0	14.0	13.8	9.0	10.4	9.7		11.2	11.5	11.4	
1 × 4		13.0	13.1	14.0	10.2	9.1	9.8	10.0	9.6	9.4	9.5	
2 × 2		12.6	14.1	14.1	10.2	10.8	10.5		12.1	10.5	11.4	
1 × 5	125		12.5			9.0						
5 × 1		12.0	13.3	13.5	10.6	9.8	9.3	9.3	11.3	12.5	11.8	
2 × 3	150	12.0	12.9	13.5	9.4	9.5	9.5		11.8	9.8	10.9	
3 × 2		11.6	13.3	13.4	9.3	9.8	9.6	9.3	12.1	11.5	11.9	
1 × 6			12.5			9.6			8.8	9.0	8.9	
6 × 1		11.2	13.0	13.1	8.4	9.5	8.9					
1 × 7	175		12.4			9.9			8.7	8.7	8.7	
7 × 1		11.2	11.8	13.0	8.4	8.6	8.5	8.5				
3 × 1	200	10.6	12.1	12.3	7.8	9.4	8.6		8.4	7.8	8.2	
1 × 8								8.7	11.1	9.1	10.2	
2 × 4		11.3	11.9	12.5	8.9	7.6	8.4		10.2	10.6	10.4	
4 × 2		10.6	12.8	12.6	8.4	9.7	9.0					
3 × 3	225	11.0	12.5	12.8	9.0	8.7	8.9	8.8	11.5	11.2	11.4	
9 × 1		9.3	10.7	10.7	8.4	9.2	8.8		8.1	8.1	8.1	
1 × 9												
10 × 1	250	8.6	10.6	10.3	7.8	9.0	8.4		7.8	7.4	7.6	
1 × 10								8.7	10.4	11.6	11.0	
5 × 2		10.9	12.0	12.5	8.9	9.0	9.0		9.6	8.8	9.2	
2 × 5			11.0			7.7						
2 × 6	300		11.2			8.4			9.5	8.8	9.2	
6 × 2		10.1	11.8	12.1	8.3	8.5	8.4					
3 × 4		10.5	11.3	12.0	8.2	7.0	7.7	8.1	7.7	10.0	8.8	
4 × 3		9.6	12.0	12.0	7.9	8.6	8.2		9.7	10.4	10.0	
7 × 2	350	10.1	10.6	12.0	8.3	7.8	8.1	9.1	9.4	9.0	8.2	
2 × 7			11.5	11.9		8.6	10.1					
3 × 5	375		10.4			7.1			7.6	6.7	7.2	
5 × 3		10.2	11.2	12.0	8.0	8.4	8.2	8.2	10.0	11.1	10.5	
2 × 8	400		10.7			8.0			9.1	7.5	8.3	
8 × 2		9.6	10.9	11.3	7.6	8.4	5.9	6.6	9.4	9.8	9.6	
4 × 4		9.7	10.8	11.3	7.6	6.7	7.2					
9 × 2	450	7.1	9.4	9.8	7.0	7.4	7.2		9.0	7.8	8.5	
2 × 9								7.7			7.8	
3 × 6		9.8	11.1	11.6	7.6	7.8	8.2					
3 × 3							7.7					
2 × 10	500			11.5			9.7				7.9	
10 × 2				9.0			6.9	8.1				
4 × 5				10.8			8.5				8.6	
5 × 4				11.2			7.2				10.0	
3 × 7	525			11.3			9.6	9.6			10.3	
7 × 3											7.5	
2 × 11	550			10.7			8.5	7.8			9.1	
11 × 2				9.0			7.1					

TABLE III—*contd*

Plot size (rows × columns)	Area in sq. ft.	Blocks running along rows						Blocks running along columns			
		C. V. before elimination			C. V. after elimination			C. V. after elimination			Average of sets 1, 2, 3 & 4
		Sets 1 & 4	Sets 2 & 3	Sets 1, 2, 3 & 4	Sets 1 & 4	Sets 2 & 3	Sets 1, 2, 3 & 4	Sets 1 & 4	Sets 2 & 3	Sets 1, 2, 3 & 4	
4 × 6 6 × 4 2 × 12 12 × 2 3 × 8 8 × 3	600		8.7	10.9 10.4 10.1 10.8 10.6		4.5	6.7 8.1 7.1 9.6 7.2			9.4 8.1 6.6	8.0
5 × 5 2 × 13 9 × 3 4 × 7 6 × 5 2 × 16 3 × 11 4 × 10	625 650 675 700 750 800 825 1000			10.6 10.4 8.5 11.0 10.4 9.9 11.1 8.2			8.7 8.1 7.0 9.2 8.1 9.2 9.0 7.6			9.8 7.6 9.3 9.3 7.2 10.6 8.6	9.8 7.6 9.3 9.3 7.2 10.6 8.6
6 × 7 3 × 14	1050			10.4 10.2			8.9 8.7			10.9	10.9
4 × 13	1300			9.8			7.4			8.7	8.7
18 × 3 9 × 6	1350			6.8 7.7			5.9 6.3				
4 × 14 8 × 7	1400			10.1 10.3			8.5 8.5			8.8	8.8
6 × 10 12 × 5	1500			10.3 8.4			8.3 6.7				
9 × 7	1575			10.7			9.0				
4 × 16 2 × 32	1600			9.1			8.3			6.2 6.4	6.3
6 × 11 6 × 13 8 × 10 9 × 9	1650 1950 2000 2025			10.1 9.6 9.7 6.6			7.7 7.3 7.4 6.5				
6 × 14 12 × 7	2100			9.5 8.6			7.9 8.0				
6 × 15 6 × 16 9 × 11 10 × 10 8 × 13	2250 2400 2475 2500 2600			9.2 9.0 10.9 7.2 8.9			8.2 8.1 7.9 7.6 6.4				
18 × 6 9 × 12	2700			5.8 6.3			5.0 6.7				
8 × 14 12 × 10 9 × 14 8 × 16 12 × 11 12 × 13	2800 3000 3150 3200 3300 3900			9.5 8.1 9.8 8.5 8.2 6.8			7.6 6.3 7.7 7.6 6.1 5.8				

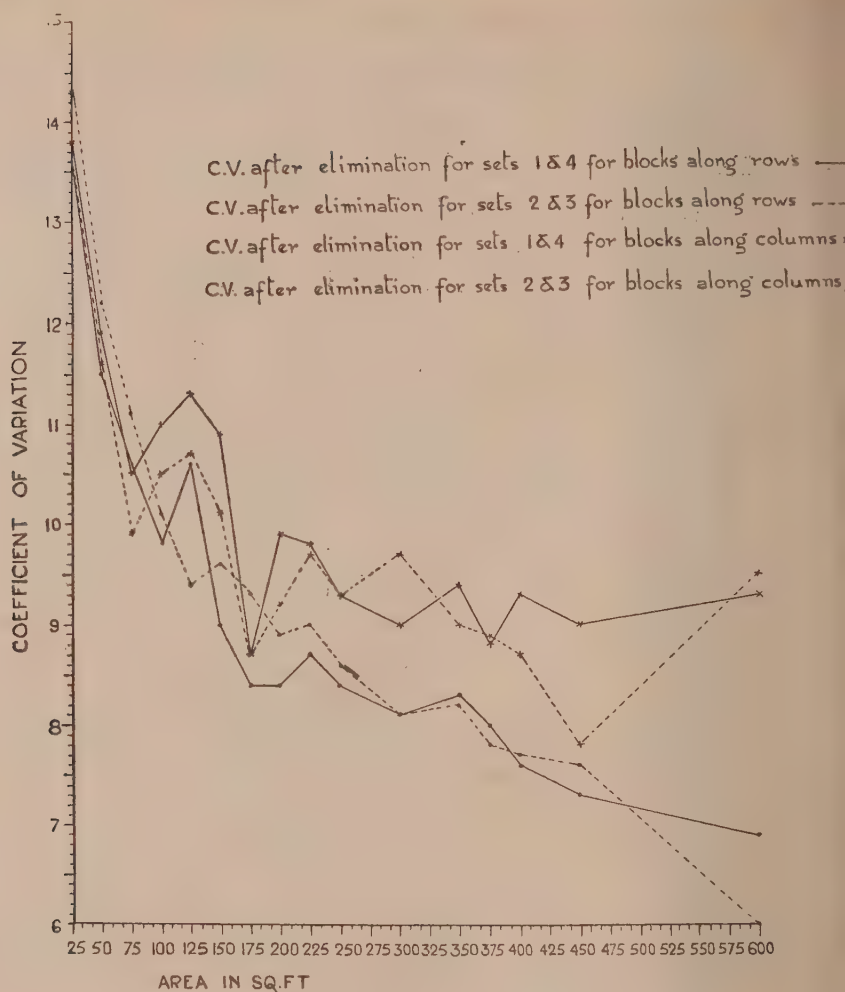


FIG. 1. Average coefficient of variation for different plot sizes

Before discussing Table III we shall make a brief survey of conclusions arrived at by other workers on the question of size of plot experiments with wheat. Mercer and Hall [1911] find that the probability of error is reduced for all practical purposes to a minimum when the size of plot is $1/50$ acre. Hall and Russell [1911] hold that each treatment should be repeated five times in plots of $1/50$ acre in size. Montgomery [1913] concludes that $5 \text{ ft.} \times 16 \text{ ft.}$ is an excellent size when plenty of land is available. Day [1920] finds that the most effective replicated block is the one that is long and narrow and has its greater dimension in the direction of greatest variation. According to Christidis [1931] the plots should be as long and narrow as possible within the limits of practical considerations. Bose [1931] says that the best plot sizes for future experiments appear to be $96 \text{ ft.} \times 4 \text{ ft.}$

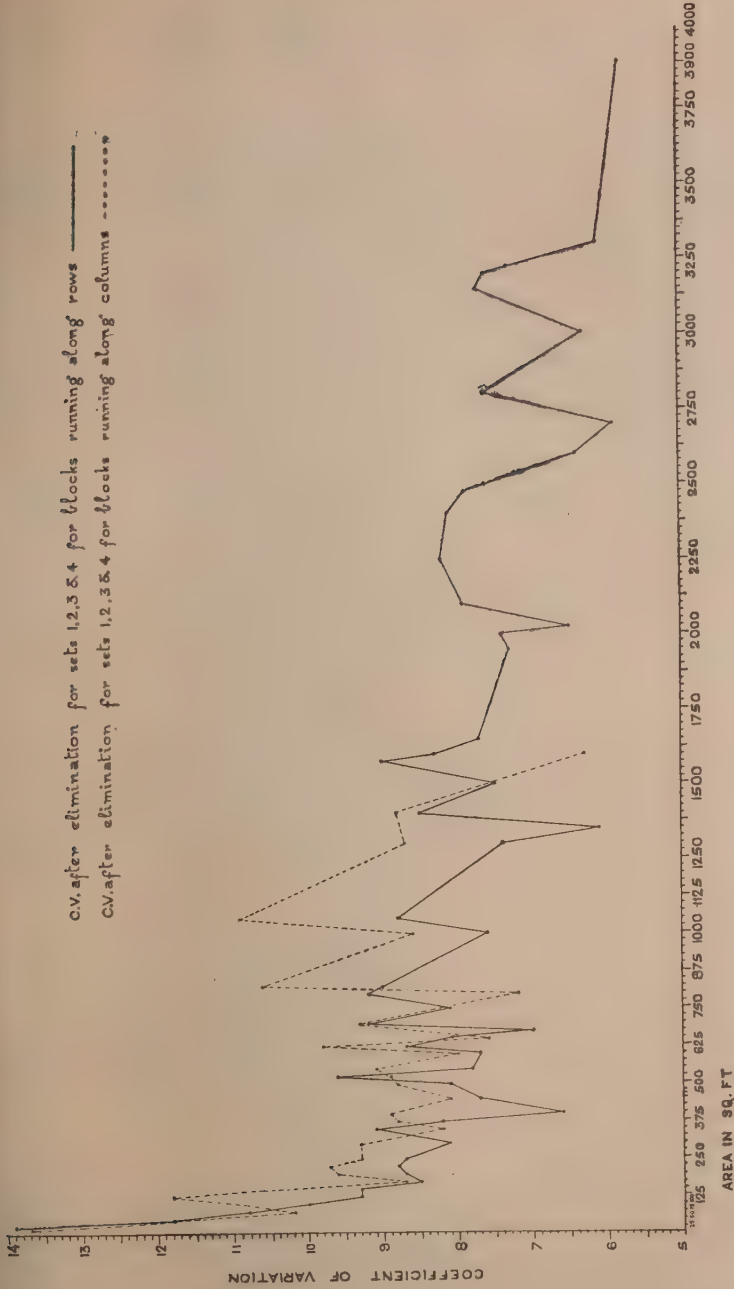


Fig. 2. Average coefficient of variation for different plot sizes

48 ft. \times 12 ft., 32 ft. \times 12 ft. and 24 ft. \times 12 ft. Long and narrow plots running across the fertility trend seem to be preferable to plots which approach a square.

Plot size and C. V. before elimination*

The examination of the C. V. before elimination is important for determining the size of sample for estimating yield by sampling. The estimation of yield must be made with as little error as possible and this can be done by fixing a sampling unit with a low error. This information is obtained from the C. V. before elimination for the different areas.

Whether we consider sets 1 and 4 or sets 2 and 3 or all the sets together the C. V. before elimination for the different plot sizes steadily diminishes with the increase in area up to 225 sq. ft. In the first two cases the C. V. of areas exceeding this size can be considered to be more or less the same with small fluctuations. On taking all the sets together there is further reduction in error when the plot size reaches about 600 sq. ft. and still more reduction for areas of 1,600 sq. ft. or more. For areas between 600 sq. ft. and 1,600 sq. ft. the error remains almost the same. As the values for larger plot sizes do not show any consistent trend, much importance cannot be attached to the low errors obtained beyond 1,600 sq. ft. Thus it appears that for estimating yield from small areas, say 5 to 10 acres, the best sampling unit, as judged from this experiment, is about 225 sq. ft. and for larger areas this may be somewhere near 600 sq. ft. or 1600 sq. ft. The area of 10 acres has been fixed on a rough basis on the assumption that the area sampled is about 5 per cent and that the number of sampling units is between 6 and 10.

There is now the question as to the method of collecting this sample unit. This sample can be taken either from one spot at random or a number of small samples can be taken from different points selected at random and then mixed up to form a composite sample. Commonsense suggests that the latter method might be preferable to the former one. However, we hoped to deal with this aspect in a subsequent paper by using the same data.

Plot size and C. V. after elimination

Taking the case of the blocks running along rows the C. V. after elimination is practically a minimum when the area of the plot is about 400 sq. ft. For blocks running along columns also there is considerable reduction in C. V. with increased plot size up to a certain point, i.e. somewhere about 450 sq. ft. For larger areas, excepting for the largest plot size, the reduction or increase in error is not so marked. It is now clear that the plot size for experiments with wheat can be fixed at about 400 sq. ft.

Shape of plots

Table IV gives the percentage efficiency ($100 \times$ ratio of variance before and after elimination) for different plot sizes. Examining this table we find that the elimination for soil heterogeneity is not so effective when the area of the plot is greater than 600 sq. ft. This table also shows that, on the whole, there is greater variation between rows than between columns.

* C. V. = coefficient of variation

TABLE IV

Percentage efficiency for different plot sizes

Plot size rows \times col- umns)	Area in sq. ft.	Blocks running along							
		Rows				Columns			
		Sets 1 & 4	Sets 2 & 3	Sets 1, 2, 3 & 4	Average for sets 1, 2, 3 & 4	Sets 1 & 4	Sets 2 & 3	Sets 1, 2, 3 & 4	Average for sets 1, 2, 3 & 4
1 \times 1	25	159	159	172	172	155	175	177	177
1 \times 2	50	150	158	171	182	174	192	202	176
2 \times 1		172	177	192		126	159	150	
1 \times 3	75	158	165	184	194	171	197	208	188
3 \times 1		174	183	203		136	160	168	
4 \times 1	100	178	182	205	197	115	121	143	167
1 \times 4		161	206	205		183	196	216	
2 \times 2		153	170	182		108	155	142	
1 \times 5	125	..	190	..	211	..	208	..	131
5 \times 1		182	182	211		113	113	131	
2 \times 3	150	164	184	203	205	100	152	139	169
3 \times 2		154	182	196		109	107	149	
1 \times 6		..	168	..		178	192	218	
6 \times 1		177	188	216		
1 \times 7	175	..	158	..	232	170	205	221	221
7 \times 1		180	185	232		
8 \times 1	200	184	165	205	208	172
1 \times 8			161	224	236	
2 \times 4		160	245	224		103	140	137	
4 \times 2		159	173	194		109	115	143	
3 \times 3	225	149	207	208	178	97	102	141	189
9 \times 1		122	135	147		
1 \times 9			172	212	231	
10 \times 1	250	122	140	153	173	168
1 \times 10			170	224	236	
5 \times 2		148	177	192		110	107	129	
2 \times 5		..	205	..		108	143	140	

TABLE IV—*contd*

Plot size (rows × columns)	Area in sq. ft.	Blocks running along							
		Rows				Columns			
		Sets 1 & 4	Sets 2 & 3	Sets 1, 2, 3 & 4	Average for sets 1, 2, 3 & 4	Sets 1 & 4	Sets 2 & 3	Sets 1, 2, 3 & 4	Average for sets 1, 2, 3 & 4
2 × 6	300	..	176	..	221	104	134	135	135
6 × 2		149	194	208		
3 × 4		161	260	242		147	95	131	
4 × 3		147	198	212		104	104	140	
7 × 2	350	150	187	220	179	208
2 × 7		..	178	138		100	137	208	
3 × 5	375	..	216	..	212	109	116	154	140
5 × 3		162	177	212		103	101	126	
2 × 8	400	..	181	..	304	116	170	184	160
8 × 2		162	168	363		
4 × 4		164	258	244		106	92	135	
9 × 2		104	162	185		
2 × 9	450	205	107	162	177	159
3 × 6		201		141	
6 × 3		165	205	228		
2 × 10		143		142	
10 × 2	500	170	178	128
4 × 5		158		117	
5 × 4		239		124	
3 × 7		139		146	
7 × 3	525	139	232	189
2 × 11	550	158	158	128	128
11 × 2		158		
4 × 6	600	..	372	..	208	87	85	127	137
6 × 4		265		
2 × 12		164		140	
12 × 2		263		
3 × 8		129		144	
8 × 3		162	174	221		
5 × 5	625	147	147	121	121
2 × 13	650	165	165	194	194
9 × 3	675	150	150
4 × 7	700	142	142	138	138
6 × 5	750	166	166
2 × 16	800	117	117	130	130
3 × 11	825	154	154	133	133
4 × 10	1000	119	119	155	155

TABLE IV—*concl'd*

Plot size (rows × columns)	Area in sq. ft.	Blocks running along							
		Rows				Columns			
		Sets 1 & 4	Sets 2 & 3	Sets 1, 2, 3 & 4	Average for sets 1, 2, 3 & 4	Sets 1 & 4	Sets 2 & 3	Sets 1, 2, 3 & 4	Average for sets 1, 2, 3 & 4
6 × 7	1050	136		
3 × 14		136	136	134	134
4 × 13	1300	174	174	125	125
18 × 3	1350	130	139
9 × 6		148		
4 × 14	1400	140		131	
8 × 7		147	144	131
6 × 10	1500	155	155
12 × 5		154		
9 × 7	1575	142	142
4 × 16	1600	121	121	127	123
2 × 32		118	
6 × 11	1650	175	175
6 × 13	1950	175	175
8 × 10	2000	173	173
9 × 9	2025	104	104
6 × 14	2100	145	131
12 × 7		116	
6 × 15	2250	125	125
6 × 16	2400	123	123
9 × 11	2475	192	192
10 × 10	2500	90	90
8 × 13	2600	195	195
18 × 6	2700	136	113
9 × 12		89			
8 × 14	2800	155	155
12 × 10	3000	164	164
9 × 14	3150	164	164
8 × 16	3200	125	125
10 × 11	3300	181	181
12 × 13	3900	136	136

Taking now the plot size 5 ft. \times 10 ft. we note from Table IV that the is greater variation between columns (i.e. across rows) than between rows thus indicating that there is greater reduction in variability when the length of the plot runs along rows, i.e. in the direction of greater variation. The above fact is borne out by a large number of other plot sizes also. Thus, for all the sizes 10 ft. \times 5 ft., 5 ft. \times 15 ft., 15 ft. \times 5 ft., 20 ft. \times 5 ft., 5 ft. \times 20 ft., 15 ft. \times 10 ft., 5 ft. \times 30 ft., 5 ft. \times 35 ft., 20 ft. \times 10 ft., 25 ft. \times 10 ft., 20 ft. \times 15 ft. and 25 ft. \times 15 ft., the length will be found to run along the direction of greater variation. This finding is in accordance with those of Day [192] and of Bose [1935]. Day finds that the variability in oblong plots is smaller than in the square ones, provided the length of the plot lies along the direction of greater change of soil fertility. Bose also finds that plots taken at right angles to the direction of the fertility gradient would probably give smaller variability than those lying along this direction.

Table V shows the relation between L/B and the C. V. after elimination. It will be seen that this table does not lead us to any definite conclusion regarding the relationship between L/B and the C. V. for different plot sizes. The evidence available is not sufficient to enable us to assume any relationship between the C. V. after elimination and L/B. We have already found that, if and when L/B is greater than 1, the length of the plot must run along the direction of greater variation.

TABLE V

C. V. after elimination for different values of L/B and areas

L/B	Dimensions of plot	C. V. after elimination	L/B	Dimensions of plot	C. V. after elimination
1.00	5 ft. \times 5 ft.	13.9	1.25	20 ft. \times 25 ft.	8.5
	10 ft. \times 10 ft.	10.5		25 ft. \times 20 ft.	7.2
	15 ft. \times 15 ft.	8.9		40 ft. \times 50 ft.	7.4
	20 ft. \times 20 ft.	7.2	1.29	45 ft. \times 35 ft.	9.0
	25 ft. \times 25 ft.	8.7			
	45 ft. \times 45 ft.	6.5	1.33	15 ft. \times 20 ft.	7.7
1.08	50 ft. \times 50 ft.	7.6		20 ft. \times 15 ft.	8.2
	60 ft. \times 65 ft.	5.8		45 ft. \times 60 ft.	6.7
1.09	60 ft. \times 55 ft.	6.1	1.50	10 ft. \times 15 ft.	9.5
1.14				15 ft. \times 10 ft.	9.6
	40 ft. \times 35 ft.	8.5		30 ft. \times 20 ft.	6.7
1.17				45 ft. \times 30 ft.	6.3
	30 ft. \times 35 ft.	8.9	1.56	45 ft. \times 70 ft.	7.7
1.20	30 ft. \times 25 ft.	8.1			
	60 ft. \times 50 ft.	6.3	1.625	40 ft. \times 65 ft.	6.4
1.22			1.67	25 ft. \times 15 ft.	8.2
	45 ft. \times 55 ft.	7.9		30 ft. \times 50 ft.	8.3

TABLE V—*contd.*

L/B	Dimensions of plot	C. V. after elimina- tion	L/B	Dimensions of plot	C. V. after elimina- tion
1.71	60 ft. × 35 ft.	8.0	3.50	35 ft. × 10 ft.	8.1
1.75	20 ft. × 35 ft.	9.2		10 ft. × 35 ft.	10.1
	40 ft. × 70 ft.	7.6		20 ft. × 70 ft.	8.5
1.83	30 ft. × 55 ft.	7.7	3.67	15 ft. × 55 ft.	9.0
2.00	5 ft. × 10 ft.	12.2	4.00	20 ft. × 5 ft.	9.7
	10 ft. × 5 ft.	11.4		5 ft. × 20 ft.	9.8
	10 ft. × 20 ft.	8.4		40 ft. × 10 ft.	5.9
	20 ft. × 10 ft.	9.0		20 ft. × 80 ft.	8.3
	15 ft. × 30 ft.	8.2	4.50	45 ft. × 10 ft.	7.2
	30 ft. × 15 ft.	7.7			
	40 ft. × 80 ft.	7.6	4.67	15 ft. × 70 ft.	8.7
2.17	30 ft. × 65 ft.	7.3	5.00	25 ft. × 5 ft.	9.3
2.33	15 ft. × 35 ft.	9.6		10 ft. × 50 ft.	9.6
	30 ft. × 70 ft.	7.9		50 ft. × 10 ft.	6.9
2.40	60 ft. × 25 ft.	6.7	5.50	10 ft. × 55 ft.	8.5
2.50	25 ft. × 10 ft.	9.0		55 ft. × 10 ft.	7.1
	20 ft. × 50 ft.	7.6	6.00	30 ft. × 5 ft.	8.9
	30 ft. × 75 ft.	8.2		10 ft. × 60 ft.	8.1
2.67	15 ft. × 40 ft.	9.6		60 ft. × 10 ft.	7.1
	40 ft. × 15 ft.	7.2	6.50	90 ft. × 15 ft.	5.9
	30 ft. × 80 ft.	8.1			
3.00	5 ft. × 15 ft.	11.2	7.00	10 ft. × 65 ft.	8.1
	15 ft. × 5 ft.	10.3		35 ft. × 5 ft.	8.5
	30 ft. × 10 ft.	8.4	8.00	40 ft. × 5 ft.	8.6
	45 ft. × 15 ft.	7.0		10 ft. × 80 ft.	9.2
	90 ft. × 30 ft.	5.0	9.00	45 ft. × 5 ft.	8.8
3.25	20 ft. × 65 ft.	7.4	10.00	50 ft. × 5 ft.	8.4

RELATIVE EFFICIENCY OF DIFFERENT LAY-OUTS

Randomized blocks versus Latin square

Doubts have been expressed as to whether Latin squares will really a smaller residual error than randomized blocks. Neyman *et al.* [1935] that 'when the size of the Latin square is increased, cases when randomized blocks are more efficient are surprisingly frequent'. 'In some when it is not so', they say 'this is due to wrong arrangement of the

randomized blocks'. Yates [1935], on the other hand, after analysing the experiments laid down at Rothamsted, finds that the efficiency of the Latin square is definitely more than that of randomized blocks. Sayer, Vaidyanathan and Iyer [1936] working with sugarcane found that Latin square is more efficient than randomized blocks, except when the latter is provided with sufficient number of replications. In the case of cotton, Hutchinson and Panse [1935] found that 'if there is sufficient knowledge to design the blocks in the most advantageous manner, randomized block can give as efficient a lay-out as Latin square.'

Table VI gives the percentage efficiencies ($100 \times$ variance before elimination/variance after elimination) for Latin square and randomized block layouts with five treatments formed from the uniformity trial data for a number of plot sizes. The percentage efficiency for blocks running along columns and rows has been taken from Table IV. For Latin squares this has been calculated by taking the ratio of the variance before elimination to the pooled residual variance of the different squares that can be formed for the particular plot size, after eliminating for the effects of rows and columns for each square.

TABLE VI

Percentage efficiency of Latin square and randomized block arrangements

Rows \times columns	Area	Percentage efficiency		
		Latin square	Blocks along rows	Blocks along columns
1 \times 1	5 ft. \times 5 ft.	250	172	177
1 \times 2	5 ft. \times 10 ft.	298	171	202
2 \times 1	10 ft. \times 5 ft.	275	192	150
1 \times 3	5 ft. \times 15 ft.	317	184	208
3 \times 1	15 ft. \times 5 ft.	336	203	168
4 \times 1	20 ft. \times 5 ft.	364	205	143
1 \times 4	5 ft. \times 20 ft.	359	205	216
2 \times 2	10 ft. \times 10 ft.	251	182	142
5 \times 1	25 ft. \times 5 ft.	250	211	131
4 \times 2	20 ft. \times 10 ft.	349	194	143
3 \times 3	15 ft. \times 15 ft.	297	208	141

Table VI shows that Latin square is more efficient than randomized blocks. It is of course realized that the usual limitations of the small number of possible arrangements for the optimum plot sizes makes it necessary to be satisfied by comparing the relative efficiencies of the designs with the optimum plot sizes.

From the above discussions it follows, as has been mentioned by Fisher and Eden [1929], that if it were possible to know beforehand that the soil heterogeneity is only in one direction, randomized blocks would be more efficient than Latin square. But it is very rarely that we know anything about the variation that is likely to occur in the field. Even in cases where uniformity trials have been conducted, it is difficult to predict the direction of the fertility gradient. Further, in majority of cases Latin square has been found to be more efficient than randomized blocks and hence Latin square is likely to prove to be more efficient than randomized blocks.

Number of treatments per block

In randomized block experiments, the elimination for soil heterogeneity is supposed to be effective when the size of the block becomes very large. The question as to the number of plots that can be included in any block of such an experiment in order to have effective elimination for soil heterogeneity is considered for a number of plot sizes in Table VII. Blocks consisting of 4, 6, 7, 8, 10, 11, 13, 15, 16 and 20 plots have been taken along rows and the percentage efficiency for different plot sizes has been given in Table VII.

TABLE VII

Percentage relative efficiency for different block sizes—blocks running along rows

Rows × columns	Area	Number of treatments per block										
		4	5	6	7	8	10	11	13	15	16	20
3 × 4	15 ft. × 20 ft.	225	242	154	200	185	129	181	151	157	159	116
4 × 4	20 ft. × 20 ft.	206	244	156	204	185	131	183	147	153	156	118
5 × 4	25 ft. × 20 ft.	219	239	143	200	178	122	157	116	148	150	109
6 × 4	30 ft. × 20 ft.	257	265	158	241	203	127	190	168	177	178	117
5 × 5	25 ft. × 25 ft.	270	147	209	131	122	149	143	149	114	110	...
7 × 4	35 ft. × 20 ft.	238	285	164	335	249	130	221	184	195	197	122
6 × 5	30 ft. × 25 ft.	308	166	242	141	128	175	172	179	126	119	...
7 × 5	35 ft. × 25 ft.	330	190	338	151	131	190	185	194	130	123	...
3 × 5	40 ft. × 25 ft.	355	176	250	147	137	169	166	172	131	124	...
4 × 7	30 ft. × 35 ft.	245	136	191	179	174	141	120
10 × 6	45 ft. × 30 ft.	121	148	101	111	98	95	96	97
12 × 5	60 ft. × 25 ft.	472	154	249	122	113	160	174	173	120	115	...

Table VII shows that the number of treatments that can be arranged a block may not exceed 13, the criterion being that the percentage efficiency, barring a few exceptions, continues to be fairly high when the number of treatments ranges from 4 to 13.

Balanced incomplete and complete randomized blocks

The method of balanced incomplete randomized blocks designed by Yates [1936] can be used for experiments involving a large number of treatments or varieties. Goulden [1937] comparing the efficiency of this type lay-out with ordinary randomized blocks concludes that, in general, incomplete block method will give increased efficiency, which is partially correlated with soil heterogeneity. He further says that if the field is very uniform, there may be loss of efficiency. But this is rather unlikely to occur on the average field.

The percentage efficiencies of the incomplete block method compared with the ordinary randomized blocks have been calculated on the basis of certain areas selected from the uniformity trial by using the formula $\frac{100 t (k-1) s_b^2}{k (t-1) s_i^2}$ for five cases, and are given in Table VIII. In the above formula t , k , s_b^2 and s_i^2 stand for the number of treatments, the number of plots per blocks, and the residual variances for the complete and incomplete randomized designs respectively.

TABLE VIII

Percentage efficiencies of balanced incomplete randomized blocks

Rows × columns	Area	Area selected for examination		No. of treat- ments	No. of replica- tions	No. of treat- ments in each block	No. of times a pair of treat- ments occurs in the whole experi- ment	No. of blocks taken	Efficien- cy
		Rows	Columns						
1×1	5 ft. × 5 ft.	8—18	21—25	11	5	5	2	11	114
3×3	15 ft. × 15 ft.	7—21	21—53	11	5	5	2	11	83
5×3	25 ft. × 15 ft.	6—25	21—59	13	4	4	1	13	57
6×4	30 ft. × 20 ft.	1—24	5—56	13	4	4	1	13	180
4×2	20 ft × 10 ft.	1—24	25—56	16	6	6	2	16	119

The percentage efficiencies for the different values of λ , t and k dealt in Table VIII have also been calculated on the basis of the results presented in Table VII by assuming s_b^2/s_i^2 to be equal to the ratio of the efficiencies of blocks having k and t treatments and taking the product of this with $\frac{100t(k-1)}{k(t-1)}$. The results obtained for the three different cases

with in Table VIII are given in Table IX.

Tables VIII and IX show that the percentage efficiency is comparatively greater for the case of 16 treatments than that for 13 or 11. Thus it appears that when the number of treatments to be experimented with is greater than 13, the balanced incomplete randomized block design is preferable to the ordinary randomized block.

TABLE IX

Percentage efficiency of incomplete randomized blocks

Rows \times columns	$\lambda=2, t=16, k=6$	$\lambda=2, t=11, k=5$	$\lambda=1, t=13, k=4$
16 \times 16	86	118	121
16 \times 11	89	117	114
16 \times 6	85	134	123
11 \times 16	79	123	99
11 \times 11	169	90	118
11 \times 6	74	113	84
6 \times 16	181	85	112
6 \times 11	244	90	111
6 \times 6	179	93	134
13 \times 13	..	100	81
13 \times 11	..	136	..
13 \times 6	192	78	177

SUMMARY AND CONCLUSIONS

A wheat uniformity trial consisting of two thousand plots each of size 5×5 ft. has been examined to obtain information on the distribution of yields for different plot sizes from different areas, the size and shape of plots, and the comparative efficiencies of 5×5 Latin squares and randomized blocks.

with five treatments in each block and also the relative efficiencies of randomized blocks having four, five, six, seven, etc. treatments per block. The efficiency of the balanced incomplete randomized blocks has also been compared with the usual randomized blocks for three cases involving 11, 13 and 16 treatments.

The following are the conclusions from the present investigation :—

(i) The distribution of yields from smaller areas is more likely to be normal than from larger areas. The value of β_1 approaches zero as the size of the plot increases. β_2 is not significantly different from 3 and generally decreases as the plot size increases. The distribution of yields approaches normality as the plot-size is increased.

(ii) For the purpose of estimating yield by sampling from fairly small areas extending from five to ten acres, the best size of the sampling plot appears to be 225 sq. ft. For larger areas, this may be increased from 225 sq. ft. to 1,600 sq. ft. The best size of plot for experiments with wheat is about 400 sq. ft. As regards the shape of plots, no relation seems to exist between L/B and the error for different plot sizes. When L/B is greater than 1, the length of the plot must run along the direction of greater variation.

(iii) In general, Latin square appears to be more efficient than randomized blocks.

(iv) The maximum number of treatments that can be arranged in a single block of a randomized block arrangement in order to have effective elimination for soil heterogeneity can be taken to be about 13. The optimum appears to be no need to have balanced incomplete block design when the number of treatments to be experimented with is 13 or less.

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Uniformity trial at Karnal with *Pusa wheat No. 114, 1936-37*
(General Area, Plot No. 38; Area of unit plot = 5 ft. x 5 ft., i.e. 25 sq. ft. Yield of grain in ounces)

Col. No. Row No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
25	17.5	18.5	18.0	18.0	17.0	20.0	18.5	17.0	14.5	14.0	14.0	15.5	14.5	20.5	15.0	15.5	12.0	13.5	16.5	12.0
24	16.5	14.5	14.0	15.0	17.0	17.0	17.0	18.5	14.5	17.0	15.0	13.5	15.5	17.5	16.5	22.0	15.5	17.5	14.0	15.5
23	13.0	15.5	15.0	15.0	14.5	17.5	17.0	16.5	15.5	15.0	16.5	17.0	14.0	17.0	14.5	19.0	15.0	15.5	15.0	12.0
22	14.0	16.0	15.0	15.5	14.5	12.5	14.5	18.0	17.0	14.5	20.0	19.5	16.0	15.5	14.5	13.5	12.0	14.0	14.0	12.5
21	15.0	15.0	16.0	17.0	16.5	14.0	15.5	16.0	18.5	16.5	15.0	14.0	16.0	13.0	16.0	14.0	15.5	13.0	14.0	14.5
20	15.0	19.0	16.0	15.5	15.0	15.5	15.5	13.0	14.5	18.0	14.5	13.5	13.5	18.0	14.5	16.5	14.0	14.5	14.0	18.0
19	14.5	17.5	18.0	17.0	19.5	16.0	18.0	15.5	17.5	16.0	16.0	13.0	15.5	17.0	18.0	15.5	12.5	15.0	15.5	14.5
18	16.0	18.0	16.0	15.0	19.0	18.0	21.5	15.5	21.0	18.5	14.0	15.0	16.5	16.5	18.5	17.0	17.0	14.0	15.0	11.0
17	16.5	19.5	19.0	23.0	21.0	19.0	23.5	21.0	20.5	20.5	16.5	14.5	14.0	13.0	18.0	17.0	19.5	14.5	15.0	16.5
16	16.0	24.0	23.0	19.5	19.5	22.5	22.0	21.5	20.5	24.0	20.5	14.0	16.5	19.5	17.5	27.0	24.0	23.0	22.0	20.0
15	15.5	23.0	18.0	17.5	20.0	21.5	19.0	23.0	22.0	17.0	22.0	18.0	19.0	19.5	18.5	22.5	19.0	21.5	17.0	18.5
14	15.5	20.0	16.0	13.0	24.0	19.5	19.5	22.5	17.0	17.0	20.0	16.5	20.0	19.5	15.5	24.0	22.0	17.5	16.5	17.0
13	17.5	22.0	20.0	18.5	22.0	20.5	26.5	22.0	21.5	20.5	22.5	21.5	18.5	23.0	18.0	23.5	20.0	19.5	17.0	21.0
12	20.0	27.0	18.0	19.5	22.0	21.5	23.5	17.0	17.5	17.0	21.0	18.0	19.0	21.5	19.0	24.5	18.0	22.0	17.0	18.0
11	22.0	16.5	16.5	21.0	20.5	19.5	25.0	21.5	24.0	16.0	17.0	18.5	21.5	23.5	18.5	15.5	18.0	22.0	17.0	18.0
10	18.5	14.0	15.5	20.5	18.0	23.5	20.0	17.5	18.0	15.0	20.5	20.0	19.0	22.0	16.5	21.0	19.5	22.0	17.0	16.5
9	15.5	14.0	15.5	22.0	19.0	20.0	23.5	18.0	18.5	19.0	24.0	19.0	19.5	19.5	15.0	18.0	19.0	16.5	16.0	15.5
8	15.0	16.5	15.0	18.0	16.0	22.0	24.0	18.5	19.5	19.5	19.0	20.0	23.5	18.0	18.5	19.0	15.5	18.0	14.5	14.5
7	14.5	19.0	16.5	19.5	21.5	19.5	21.0	20.0	20.0	20.5	19.5	22.0	23.0	21.0	17.0	22.0	21.0	20.0	17.0	12.0
6	16.0	12.0	14.0	16.5	15.0	15.0	18.0	15.5	18.0	15.0	16.0	15.5	15.5	12.0	12.0	13.0	20.0	18.5	15.5	21.5
5	16.5	16.5	13.5	19.0	17.0	12.0	20.0	16.5	23.0	17.0	16.0	14.5	14.0	18.0	15.5	15.5	13.5	14.5	14.5	16.0
4	15.0	16.5	16.5	18.0	16.0	14.0	17.5	17.0	18.0	14.0	14.5	19.0	19.5	20.0	16.0	13.0	11.0	19.0	15.0	12.0
3	19.5	17.0	17.5	18.0	17.0	15.0	18.5	17.5	18.5	16.0	13.0	18.0	19.5	15.5	19.5	16.0	15.5	14.0	16.5	13.0
2	17.5	17.5	17.0	18.0	21.5	16.0	17.0	16.0	17.0	15.5	16.0	17.5	19.0	18.5	19.5	18.0	15.5	16.0	18.0	16.5
1	24.0	15.5	15.0	15.5	17.5	15.0	15.0	19.5	17.0	16.5	12.5	17.5	14.0	15.5	13.5	13.0	21.5	14.0	13.5	16.0

APPENDIX—*contd*

Row No.	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
25	14.0	12.0	13.0	12.0	13.0	12.5	11.5	14.5	15.5	12.5	15.5	15.0	17.0	13.5	13.5	16.5	14.0	19.5	18.5	15.0
24	14.5	13.0	12.0	14.0	9.5	12.5	17.5	17.0	13.0	16.5	13.5	14.0	15.5	14.0	13.0	15.0	16.5	18.0	16.0	15.0
23	10.5	10.5	12.0	15.5	12.5	13.5	13.5	14.0	14.0	17.0	12.0	14.0	13.0	14.5	12.5	13.5	15.0	16.5	13.5	15.5
22	11.5	12.0	15.5	13.0	13.0	12.0	15.0	14.5	12.0	17.0	12.0	12.5	15.0	13.5	11.0	14.5	14.0	18.5	13.0	15.0
21	16.0	12.5	12.0	16.0	15.5	15.5	15.5	13.0	12.0	14.0	12.5	15.5	15.5	16.0	14.0	14.5	15.5	18.0	15.5	16.0
20	13.0	12.5	13.5	15.5	13.0	14.5	15.0	16.5	13.5	13.0	14.0	13.0	10.5	13.0	12.5	11.5	14.0	14.5	14.5	13.5
19	14.5	15.0	15.0	18.5	15.0	14.0	17.0	18.5	18.0	15.5	13.0	14.0	16.0	15.0	14.0	15.0	14.0	14.5	14.5	17.0
18	17.5	15.0	15.5	17.5	16.5	17.0	14.5	17.5	17.0	17.0	14.0	13.5	15.5	14.0	12.0	15.0	14.5	16.0	15.0	16.5
17	17.5	13.0	16.0	18.0	20.0	15.5	19.5	18.5	17.0	20.0	15.0	15.0	15.0	14.5	14.5	17.0	15.0	19.5	17.0	16.0
16	19.0	20.0	17.0	17.5	13.0	15.0	17.5	19.0	20.0	16.5	14.0	17.0	15.0	13.5	16.0	16.0	17.5	14.5	15.0	16.0
15	19.0	17.0	15.0	15.5	16.0	16.0	17.0	19.0	20.0	16.5	14.5	16.0	13.0	15.5	14.5	13.5	20.0	12.5	16.0	17.0
14	20.5	18.0	15.5	17.5	13.0	14.5	13.0	15.5	16.0	14.5	15.5	13.0	13.0	13.0	16.0	20.5	20.5	17.5	12.5	14.0
13	22.0	22.0	21.5	19.5	17.0	12.0	13.0	20.5	23.5	16.0	14.0	13.0	14.0	17.0	19.0	22.0	22.0	25.0	13.5	20.0
12	20.5	13.0	22.5	18.0	15.5	13.0	13.0	18.0	19.0	14.5	15.5	13.5	16.0	19.0	15.5	19.0	19.0	20.0	20.0	15.5
11	16.5	21.0	13.5	17.0	17.0	12.0	15.5	21.0	23.0	22.0	18.5	16.0	16.0	14.0	13.5	17.5	21.5	19.5	14.5	13.5
10	13.5	18.5	12.0	15.5	14.5	12.5	15.5	21.0	19.5	18.0	15.5	16.0	14.0	18.5	17.5	19.5	18.5	21.5	15.0	19.5
9	19.0	21.0	16.0	19.5	16.0	15.0	14.0	14.0	13.5	15.0	12.0	15.0	14.0	14.5	16.0	19.0	18.5	17.0	17.0	19.5
8	10.5	16.0	13.5	15.5	16.0	14.5	17.0	20.5	19.0	13.0	16.0	12.0	12.0	16.0	17.0	15.5	14.0	13.5	16.0	17.0
7	10.5	17.0	14.0	15.0	15.5	15.5	19.5	23.0	19.0	15.0	17.0	15.5	13.0	15.5	12.5	15.5	14.5	13.0	9.5	17.0
6	19.0	20.5	18.0	21.5	21.0	19.0	19.0	20.5	18.0	15.0	18.0	14.5	15.5	16.0	18.0	16.5	16.0	20.0	13.0	16.0
5	19.5	21.5	13.0	17.5	17.5	15.0	19.5	16.5	20.5	22.0	20.5	17.5	17.0	17.0	19.0	20.5	17.0	20.5	15.0	18.0
4	16.0	19.0	16.5	15.5	14.5	15.0	21.0	19.0	14.5	12.0	16.5	14.0	13.0	12.0	14.5	20.0	14.5	17.0	12.0	13.5
3	14.0	15.5	13.5	18.0	15.5	12.0	23.5	18.5	13.0	10.0	15.0	14.5	12.0	14.0	17.0	17.0	15.5	18.5	10.5	16.0
2	16.0	15.0	8.0	12.5	14.5	14.0	24.5	20.5	12.0	10.0	14.0	10.0	11.0	16.0	13.5	14.5	13.5	16.0	13.0	16.5

Row No.	Col. No.	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
25		15.0	12.5	13.5	15.0	13.0	11.5	15.5	16.0	17.0	19.0	20.0	17.5	17.0	17.0	17.5	18.0	16.5	14.0	17.0	16.5
24		14.5	10.0	13.0	13.0	16.5	14.5	10.0	15.0	12.5	15.5	17.0	14.0	15.5	15.0	13.0	12.0	14.5	11.5	16.5	15.5
23		11.5	11.0	14.5	13.0	12.5	17.0	14.0	11.0	14.0	13.0	11.0	15.0	15.0	18.0	14.5	16.0	14.5	14.0	12.5	14.0
22		14.0	11.0	17.0	12.0	14.0	13.0	12.0	15.5	12.5	14.0	13.5	14.0	13.0	15.5	15.5	12.5	13.0	12.0	12.5	14.0
21		13.0	11.0	16.5	14.0	15.5	16.0	14.0	15.0	12.5	15.0	14.0	13.0	17.0	13.0	15.0	14.0	12.5	11.0	15.5	16.0
20		13.5	10.5	15.5	16.0	15.5	13.5	15.5	13.0	15.0	13.5	11.0	12.0	16.5	14.5	14.0	13.5	16.0	13.5	13.5	14.0
19		17.0	13.5	14.0	13.5	16.0	18.5	13.0	17.5	15.0	15.5	11.0	16.0	15.0	13.5	15.5	17.0	15.0	13.0	15.0	20.5
18		16.0	14.0	19.5	13.5	17.0	16.0	13.5	11.0	16.0	15.5	13.5	16.0	16.5	16.5	16.0	16.0	13.0	14.0	19.0	17.0
17		17.0	14.5	17.5	18.0	17.5	18.5	15.5	14.5	14.5	14.0	11.0	16.0	15.0	14.0	15.0	13.5	15.5	17.5	18.0	13.5
16		15.5	14.0	21.0	15.5	18.0	18.5	13.0	12.5	12.5	14.5	14.0	15.0	15.5	15.5	19.0	16.5	16.0	14.5	20.0	17.0
15		16.0	11.0	15.0	17.5	15.0	16.5	13.0	11.5	16.0	13.5	12.5	15.5	15.0	17.0	19.5	15.5	17.0	16.0	17.5	18.0
14		16.5	14.0	13.0	13.5	17.0	13.0	15.0	12.0	13.0	14.5	12.5	11.0	16.0	12.5	16.0	14.5	13.5	15.5	15.5	19.5
13		15.0	14.0	13.0	13.0	20.5	19.0	14.0	14.5	12.0	15.5	12.5	12.0	14.5	18.0	22.0	14.5	19.0	15.0	19.0	19.5
12		22.0	17.5	22.5	18.5	18.5	20.5	15.5	15.0	11.0	13.5	13.5	13.5	17.5	19.0	19.5	19.5	19.5	16.5	16.0	16.0
11		15.5	13.0	14.0	19.5	20.5	19.0	17.0	15.0	17.5	19.5	16.0	15.0	17.5	16.0	17.0	19.5	13.0	16.0	16.0	13.5
10		16.0	15.0	19.5	17.0	17.5	20.5	19.5	17.0	13.5	20.5	19.0	16.5	16.5	16.5	23.5	18.0	13.5	12.5	17.0	20.5
9		13.0	14.0	17.0	20.5	20.5	22.0	18.0	16.5	19.0	16.0	15.5	15.5	16.0	16.5	23.5	15.5	15.5	16.0	19.0	17.5
8		16.0	14.5	20.0	20.0	18.0	16.5	12.5	16.0	15.0	15.0	13.5	14.0	14.5	16.5	23.5	18.5	17.0	15.0	17.0	20.0
7		13.0	13.5	13.5	14.0	16.0	17.0	17.5	15.5	15.5	14.0	14.0	15.0	13.0	19.0	21.0	19.0	18.0	18.5	15.0	17.0
6		15.5	12.0	20.0	18.0	20.5	13.5	17.0	16.0	17.0	14.0	17.5	15.5	17.0	17.0	21.0	19.5	19.0	16.0	19.0	13.0
5		21.0	16.0	23.0	21.0	18.0	21.5	21.5	18.0	22.0	21.5	19.5	15.5	16.5	19.0	20.0	21.0	18.5	15.0	16.0	17.0
4		13.0	14.0	22.5	19.5	20.0	26.5	22.5	20.0	25.5	20.5	20.0	18.0	19.0	17.5	19.0	16.0	17.5	16.0	13.0	14.5
3		16.5	13.0	18.0	17.5	19.5	19.0	17.0	18.5	21.5	22.0	27.0	21.0	18.5	22.5	13.5	15.0	14.0	12.0	17.0	20.5
2		14.0	15.5	21.0	25.0	21.5	21.0	23.0	16.0	21.5	28.0	19.0	22.0	19.0	24.0	19.5	13.5	13.0	18.0	15.5	13.5
1		15.5	15.0	17.5	13.0	17.5	16.0	19.0	16.0	20.0	17.0	19.0	20.0	21.5	17.5	20.0	15.0	14.5	13.5	14.5	16.5

APPENDIX—*concl'd*

Col. No.	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80
Row No.																				
25	14.5	15.5	19.0	22.0	22.0	25.0	27.5	27.5	24.0	21.0	24.0	26.0	19.0	18.5	24.0	21.5	24.5	20.0	20.5	23.0
24	16.5	11.0	15.5	15.5	19.0	20.0	22.0	20.5	21.0	22.5	27.0	21.0	16.5	22.0	19.5	20.0	24.0	25.5	22.5	21.0
23	14.5	14.0	16.5	15.0	14.5	16.5	19.5	18.0	20.0	16.5	17.0	18.0	15.5	18.0	22.0	17.0	18.0	20.5	16.5	17.5
22	12.0	12.5	16.5	16.0	14.5	19.0	21.0	23.5	19.5	15.0	17.5	16.5	15.5	16.5	21.5	24.5	18.0	19.5	17.5	18.0
21	14.5	13.5	15.5	18.5	17.5	20.5	22.0	19.0	24.0	23.5	25.5	19.0	16.0	16.0	17.5	19.5	21.0	23.0	18.0	16.5
20	15.0	15.0	16.5	17.0	16.5	17.0	16.5	18.5	15.5	19.0	23.0	20.5	21.5	18.0	15.5	15.0	15.5	18.0	17.0	16.5
19	14.5	16.5	20.5	18.5	18.0	15.0	20.5	16.5	18.5	19.0	18.5	16.5	18.5	20.5	23.0	20.5	19.5	14.5	15.0	17.0
18	15.5	17.0	20.0	17.5	18.5	19.5	24.0	18.0	15.5	17.0	16.5	14.0	14.0	12.0	19.5	19.0	19.0	21.5	13.5	19.5
17	19.5	18.5	18.5	23.5	22.0	26.0	19.5	22.5	22.5	19.0	17.0	21.0	17.5	15.5	19.5	17.0	16.5	24.0	16.5	16.0
16	15.5	16.0	20.0	21.5	19.0	21.5	19.5	25.5	22.0	20.0	17.0	21.5	21.0	17.0	21.0	20.5	16.5	23.5	20.5	21.0
15	19.5	18.0	17.0	20.5	17.0	21.0	19.5	23.0	19.5	15.5	15.0	15.5	18.5	16.0	18.5	23.5	19.0	18.0	15.5	23.0
14	17.5	18.5	17.0	23.0	20.5	19.5	18.5	18.0	9.5	8.0	8.5	14.0	15.0	17.0	19.0	15.5	19.0	18.0	15.0	18.5
13	15.0	17.5	22.0	21.0	22.5	19.0	18.0	14.0	7.5	14.0	13.0	15.0	14.5	18.5	18.0	16.0	14.5	17.5	15.0	20.5
12	19.5	16.5	18.5	18.5	22.5	23.5	21.0	17.5	10.5	12.5	18.0	16.0	17.5	15.5	20.0	21.0	16.5	19.5	20.0	20.0
11	17.0	19.5	24.5	22.5	20.5	25.0	20.5	18.0	19.5	14.5	16.0	21.0	18.0	16.5	17.5	18.0	17.5	19.0	16.5	21.0
10	15.5	19.0	17.5	19.5	23.0	20.0	18.0	17.5	19.0	16.0	18.0	14.5	15.5	15.5	16.0	16.0	15.0	20.0	18.0	23.5
9	18.0	20.5	19.5	19.0	16.5	18.0	18.5	17.5	14.5	19.5	14.0	18.0	18.0	18.0	16.5	18.0	17.0	20.0	20.0	18.0
8	15.0	15.0	15.5	21.0	18.0	22.5	18.5	19.0	18.5	16.5	15.5	15.0	17.0	20.0	20.0	15.0	18.0	24.5	17.5	21.0
7	18.0	16.5	15.0	16.0	17.0	15.5	18.0	19.5	16.5	18.0	20.0	19.0	19.0	19.5	21.0	18.5	21.0	26.0	17.5	23.5
6	15.5	21.0	17.0	17.5	19.5	19.5	18.0	16.0	15.5	12.0	14.0	12.5	16.5	16.5	15.0	18.0	18.0	22.0	22.0	22.0
5	14.5	20.0	16.0	17.5	18.5	19.0	18.5	19.0	13.0	14.5	14.5	15.5	16.5	17.5	16.0	18.5	18.0	20.5	20.0	21.0
4	17.0	16.0	15.5	16.5	20.0	15.5	18.0	18.0	16.0	13.5	12.5	15.0	15.5	16.5	18.0	17.5	15.5	20.0	20.0	23.5
3	13.5	16.5	16.0	16.0	20.5	20.5	18.5	15.5	13.5	17.0	18.0	17.0	15.0	14.5	17.0	15.0	14.0	16.5	16.5	24.0
2	14.5	10.5	16.0	19.5	24.0	20.0	18.0	16.0	15.5	16.5	16.0	15.5	15.5	17.5	16.0	15.0	14.5	18.0	19.0	22.5
1	14.0	11.0	14.0	18.5	15.5	15.5	15.0	16.0	18.5	14.0	13.0	15.0	13.0	13.0	14.0	16.0	14.0	18.0	18.0	15.5

II. BALANCED VERSUS RANDOMIZED ARRANGEMENTS

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a paper entitled 'Co-operation in large scale experiments' Gosset [1936] expressed the opinion that 'the advantages of artificial randomization are fully offset by an increased error when compared to balanced arrangements.' Fisher, who does not agree with this view, carried out some investigations with a uniformity trial data in collaboration with Barbaek [1936] and found that random arrangements would give smaller errors than systematic ones. Gosset [1937] examined this point in detail from the practical and the theoretical points of view and has come to the conclusion that balanced arrangements are likely to be more accurate than random designs. In support of his conclusion he has quoted Hudson who, as a result of some investigations on the uniformity trial data of Mercer and Hall [1911], Kalamkar [1932] and Immer [1932], found the balanced arrangements to be more accurate than the random arrangements. Pearson [1938] applying the power tests evolved by Neyman himself [1936] to some of the results obtained by Hudson has shown that Gosset's view is likely to be more accurate than that of Fisher. Yates [1938] pointed out that Hudson's results are of little interest for the following reasons:—

- (i) Only three uniformity trials had been used ;
- (ii) The plots in most of the arrangements were only one or two rows wide ;
- (iii) The arrangements used by Hudson were randomized blocks for random arrangements and Latin squares for balanced arrangements. Such a comparison was not fair in view of the fact that Latin squares are more accurate than randomized blocks.

After some further careful analysis he came to the conclusion that 'in cases where Latin square designs can be used and in many cases where randomized blocks have to be employed, the gain in accuracy with the systematic arrangements is not likely to be sufficiently great as to outweigh the disadvantages to which the systematic designs are subject.'

In order to have a correct idea of the relative merits of the two designs, it is necessary to examine the results that would be obtained from other uniformity trials after taking into consideration some of the many alternative arrangements that can be had for both the designs. In this paper the wheat uniformity trial data discussed in part I, have been used to examine the relative accuracy of random and systematic designs from a more comprehensive

point of view for experiments involving four, five, six and seven treatments with six, six, eight and six replications respectively.

MATERIAL

The material used in this investigation consists of certain portion of wheat uniformity trial detailed in Tables I-IV.

TABLE I

Four treatments with 6 replications each plot—3 rows \times 3 columns
(Yields in ounces)

Row No.	Column No.	64—66	67—69	70—72	73—
19—21		158·5	171·0	184·5	169
16—18		189·0	189·0	163·0	157
13—15		184·0	147·5	118·5	155
10—12		195·0	161·5	146·5	152
7—9		163·5	160·5	155·5	169
4—6		163·5	152·0	124·5	147

TABLE II

Five treatments with 6 replications each plot—3 rows \times 3 columns
(Yields in ounces)

Row No.	Column No.	45—47	48—50	51—53	54—56	57—
19—21		137·5	132·0	125·5	130·0	125
16—18		147·5	125·0	132·5	142·0	152
13—15		143·0	122·5	121·5	149·5	153
10—12		168·5	147·5	150·0	168·5	150
7—9		158·0	142·5	131·0	173·0	151
4—6		186·0	174·5	158·5	170·0	155

TABLE III

Six treatments with 8 replications each plot—3 rows \times 3 columns
(Yields in ounces)

Row No.	Column No.	1—3	4—6	7—9	10—12	13—15	16—18
2—24		133.5	138.5	148.5	148.0	141.0	144.0
9—21		146.0	146.0	144.0	136.5	141.5	130.5
3—18		168.0	176.5	187.0	157.5	150.0	173.0
3—15		167.5	176.5	193.0	175.0	171.5	189.5
0—12		168.0	186.0	184.0	163.0	180.5	181.0
7—9		141.5	177.5	183.0	182.5	175.0	169.0
4—6		136.5	142.5	163.5	141.5	142.5	138.0
1—3		160.5	153.5	156.0	142.5	154.5	143.5

TABLE IV

Seven treatments with 6 replications each plot—3 rows \times 4 columns
(Yields in ounces)

Row No.	Column No.	21—24	25—28	29—32	33—36	37—40	41—44	45—48
0—22		168.0	173.0	166.0	161.5	180.0	164.0	172.5
7—19		198.0	203.5	189.0	177.5	189.5	188.0	188.5
4—16		211.5	200.5	189.5	187.5	193.0	187.5	175.0
1—13		232.0	197.5	213.5	211.0	224.0	207.5	209.0
8—10		195.5	190.5	183.0	191.0	208.5	207.5	214.5
5—7		212.0	221.5	212.5	196.0	189.5	205.5	217.0

METHODS

Four treatments and six replications

(i) *Random arrangements.*—Three hundred random arrangements were formed by superposing four hypothetical treatments, A, B, C and D on a random basis on the data presented in Table I by considering the blocks to lie

along rows. It can be easily seen that the sum of squares for treatments plus residual error is a constant. The error for the treatments is different for different arrangements and has been calculated from the hypothetical treatment totals computed in the manner described below :—

Twenty-four identical blank cards were divided into six groups, each group having four cards. The figures in the six rows of Table I were entered in the six groups of cards. Each group of cards was then thoroughly shuffled and the dummy treatments A, B, C and D were superposed on the 1st, 2nd, 3rd and 4th cards respectively, for all the six groups of cards. The treatment totals were now got by adding up all the A's, B's, C's and D's. The sum of squares for the treatments and the residual error corresponding to the first random arrangement were calculated on the basis of these totals. The above process was repeated 300 times and the treatment and the residual errors were thus obtained for 300 random samples.

(ii) *Balanced arrangements*.—As in the case of randomized arrangements there are different ways of balancing any experiment, the only difference being that the number of arrangements that can be formed on a random fashion is much more than that on balanced basis. The treatment and the residual errors have been calculated for the arrangements given below for the case of four treatments :—

				(1)				(2)			
A	B	C	D	A	B	C	D	A	B	C	D
D	C	B	A	B	C	D	A	D	A	B	C
A	B	C	D	C	D	A	B	C	D	A	B
D	C	B	A	D	A	B	C	B	C	D	A
A	B	C	D	A	B	C	D	A	B	C	D
D	C	B	A	B	C	D	A	D	A	B	C

Chart 1. (Balancing has been effected between two rows, and the same arrangement of the treatments has been repeated in the third and fifth rows)

Chart 2. (Formed on the basis of the two diagonal squares)

A	D	C	B
B	C	D	A
C	B	A	D
D	A	B	C
A	D	C	B
B	C	D	A

Chart 3. (Formed on the basis of Latin squares by effecting balance between two rows and two columns)

The sum of squares for treatments for any of the designs shown in chart 1, 2 and 3 is independent of their arrangements in the first row so long as the scheme of balancing is as explained in the corresponding designs. If T_1 , T_2 , T_3 and T_4 are the treatment totals for A, B, C and D respectively for a particular arrangement of the treatments in the first row (for any of the designs shown

charts 1, 2 and 3), their totals for any other permutation of the first row will also be the same, the only difference being that T_1 , instead of being the total for treatment A, will now be the total for some other treatment, T_2 will be the total for either B or some other treatment, and so on. Thus, whatever the permutation of the treatments in the first row, their totals remain the same but get themselves allotted in different ways. Hence the sum of squares for the dummy treatments is fixed so long as the scheme of balancing is fixed.

In addition to the designs described above, 25 more arrangements were made by balancing two rows and by having different treatment arrangements in the first, third and fifth rows as indicated in chart 4.

A	B	C	D	}	(a)
D	C	B	A		
D	B	A	C	}	(b)
C	A	B	D		
B	C	D	A	}	(c)
A	D	C	B		

Chart 4. (Balancing effected between two rows with the treatment arrangements in a , b and c at random)

The mean and the variance of the treatment errors (i.e. variance for treatments) of the 25 balanced arrangements have been compared with those of the 300 random ones.

The calculation of the treatment sum of squares for each of the balanced designs of the type shown in chart 4 was done as follows:—

The treatment totals for the design shown in chart 4 are the sum of their respective totals for sections (a), (b) and (c). Let the treatment totals for section (a) be t_{1a} , t_{2a} , t_{3a} and t_{4a} for A, B, C and D respectively. For any other arrangement of the treatments in this section the totals will still be t_{1a} , t_{2a} , t_{3a} and t_{4a} but get allotted to different treatments in accordance with the permutation of the treatments in that section. A similar property holds good for sections (b) and (c) also. In view of this property, the treatment totals corresponding to any arrangement of the type shown in chart 4 can be calculated by taking the sectional totals t_{1a} , t_{2a} , t_{3a} , t_{4a} ; t_{1b} , t_{2b} , t_{3b} , t_{4b} ; t_{1c} , t_{2c} , t_{3c} , t_{4c} and allotting them at random to A, B, C and D in each section and then adding up the A's, B's, C's and D's. This was done by writing down three sets of totals in three groups of identical cards, each group having four cards. Each of the three groups was thoroughly shuffled and the treatments A, B, C and D were allotted to the first, second, third and fourth cards. The totals of the treatments were now computed and the treatment variance was calculated on the basis of these totals. Such a procedure was repeated 25 times and the variances for treatments corresponding to 25 balanced arrangements of the type shown in chart 4 were calculated.

Five treatments and six replications

(i) *Randomized arrangements.*—The treatment and the residual errors for randomized arrangements were calculated on the same lines as described for five treatments.

(ii) *Balanced arrangements*.—The following systematic or balanced arrangements have been compared with the 400 random arrangements mentioned above.

A	B	C	D	E
E	D	C	B	A
A	B	C	D	E
E	D	C	B	A
A	B	C	D	E
E	D	C	B	A

Chart 5. (Formed on the same lines as indicated in chart 1)

(1)					(2)				
A	B	C	D	E	A	B	C	D	E
B	C	D	E	A	E	A	B	C	D
C	D	E	A	B	D	E	A	B	C
D	E	A	B	C	C	D	E	A	B
E	A	B	C	D	B	C	D	E	A
A	B	C	D	E	A	B	C	D	E

Chart 6. (Formed on the same lines as shown in chart 2)

(1)					(2)				
A	B	C	D	E	A	B	C	D	E
D	E	A	B	C	C	D	E	A	B
B	C	D	E	A	E	A	B	C	D
E	A	B	C	D	B	C	D	E	A
C	D	E	A	B	D	E	A	B	C
A	B	C	D	E	A	B	C	D	E

Chart 7. (Formed on the basis of Knut Vik squares)

The mean and the variance of the treatment errors of 25 arrangements balanced on lines similar to that described for four treatments were also calculated.

Six treatments and eight replications

(i) *Random arrangements*.—Four hundred random arrangements were taken as described before.

(ii) *Balanced arrangements*.—The following systematic or balanced arrangements have been taken in this case.

A	B	C	D	E	F
F	E	D	C	B	A
A	B	C	D	E	F
F	E	D	C	B	A
A	B	C	D	E	F
F	E	D	C	B	A
A	B	C	D	E	F
F	E	D	C	B	A

Chart 8. (Arrangement is similar to chart 1)

(1)						(2)					
A	B	C	D	E	F	A	B	C	D	E	F
B	C	D	E	F	A	F	A	B	C	D	E
C	D	E	F	A	B	E	F	A	B	C	D
D	E	F	A	B	C	D	E	F	A	B	C
E	F	A	B	C	D	C	D	E	F	A	B
F	A	B	C	D	E	B	C	D	E	F	A
A	B	C	D	E	F	A	B	C	D	E	F
B	C	D	E	F	A	F	A	B	C	D	E

Chart 9. (Arrangement based on diagonal squares)

(1)	(2)	(3)	(4)
{ A B C D E F	A B C D E F	A B C D E F	A B C D E F
{ F E D C B A	F E D C B A	F E D C B A	F E D C B A
{ C F B E A D	B C A F D E	B A E F C D	C F A B D E
{ D A E B F C	E D F A C B	D C F E A B	E D B A F C
{ B C A F D E	C F B E A D	C F A B D E	B A E F C D
{ E D F A C B	D A E B F C	E D B A F C	D C F E A B
{ A B C D E F	A B C D E F	A B C D E F	A B C D E F
{ F E D C B A	F E D C B A	F E D C B A	F E D C B A

Chart 10. (Arrangement based on two Latin squares)

Excepting for an interchange between the positions of *b* and *c*, arrangements (1) and (2) are the same. Similar is the case with (3) and (4) also.

In addition to the above, as in other cases, the means and the variances of the treatment errors of 25 arrangements balanced as shown in chart 10 are also calculated.

Seven treatments and six replications

(i) *Randomized arrangements*.—Here also, the treatment and the residual errors were calculated for 400 random arrangements.

(ii) *Balanced arrangements*.—The following systematic or balanced arrangements have been compared with the 400 random arrangements:

A	B	C	D	E	F	G
G	F	E	D	C	B	A
A	B	C	D	E	F	G
G	F	E	D	C	B	A
A	B	C	D	E	F	G
G	F	E	D	C	B	A

Chart 11. (Arrangement similar to that of chart 1)

(1)							(2)						
A	B	C	D	E	F	G	A	B	C	D	E	F	G
B	C	D	E	F	G	A	G	A	B	C	D	E	F
C	D	E	F	G	A	B	F	G	A	B	C	D	E
D	E	F	G	A	B	C	E	F	G	A	B	C	D
E	F	G	A	B	C	D	D	E	F	G	A	B	C
F	G	A	B	C	D	E	C	D	E	F	G	A	B

Chart 12. (Arranged on the basis of diagonal squares)

(1)							(2)						
A	B	C	D	E	F	G	A	B	C	D	E	F	G
F	G	A	B	C	D	E	C	D	E	F	G	A	B
D	E	F	G	A	B	C	E	F	G	A	B	C	D
B	C	D	E	F	G	A	G	A	B	C	D	E	F
G	A	B	C	D	E	F	B	C	D	E	F	G	A
E	F	G	A	B	C	D	D	E	F	G	A	B	C

Chart 13. (Arranged on the basis of Knut Vik squares)

Like the other three cases, the mean and the variance of the treatment errors were calculated for 25 arrangements of the type shown in chart 4 the same method as that described for four treatments.

RESULTS AND DISCUSSIONS

The residual and the treatment errors of the different types of balanced arrangements shown above have been compared with those of the randomized arrangements and the results are given below in the succeeding tables.

Table V gives the numbers of randomized arrangements having greater and less residual variance than that for balanced arrangement of the type shown in chart 1.

TABLE V

Comparison between randomized arrangements and balanced arrangements shown in chart 1

No. of treatments	No. of replications	No. of samples taken	No. of random samples with residual variance	
			Greater than chart 1	Less than chart 1
4	6	300	240	60
5	6	400	357	43
6	8	400	238	162
7	6	400	314	86

From Table V it is clear that balanced arrangements of the type indicated in chart 1 are likely to give less residual variance than randomized designs and hence comparisons based on random arrangements are likely to be more reliable than that of chart 1.

Table VI shows the numbers of randomized arrangements with greater as well as with less residual variance as compared with the arrangements based on diagonal squares.

TABLE VI

Comparison between random arrangements and diagonal squares

No. of treatments	No. of random samples with residual variance			
	Greater than chart 2 (1)	Less than chart 2 (1)	Greater than chart 2 (2)	Less than chart 2 (2)
4	199	101	56	244
5	192	208	7	393
6	159	241	240	160
7	7	393	77	323

Table VI shows that for seven treatments both the arrangements based on diagonal squares are likely to give greater residual variance than that for random arrangements. In the case of four, five and six treatments the findings are not consistent. For four treatments random arrangements are likely to give greater accuracy than that shown in chart 2 (1), while the one indicated in chart 2 (2) is likely to give more reliable information than random ones. In the case of six treatments this position is reversed. From this, it is clear that we are not sure of the exact type of balance to be effected to get greater accuracy. However, taking the eight diagonal squares, the residual error for six of the arrangements is likely to be greater than that for random arrangements.

Table VII gives the numbers of random arrangements having greater and less residual variance than that for Knut Vik squares.

TABLE VII

Comparison of random arrangements and Knut Vik squares

No. of treatments	No. of random arrangements with residual variance			
	Greater than chart 7 (1)	Less than chart 7 (1)	Greater than chart 7 (2)	Less than chart 7 (2)
5	226	174	103	297
7	375	25	73	328

Here again, the results are not consistent. Of the two arrangements shown in chart 7 (1) and 7 (2), the latter is likely to give greater accuracy than the random designs, while in the case of the former the position is just the reverse. We are thus faced with the same difficulty as in the case of diagonal squares.

Table VIII shows the numbers of random arrangements with greater as well as with less residual variance than that for arrangements given in chart 10 for six treatments.

TABLE VIII

Comparison between random arrangements and balanced Latin squares for six treatments with eight replications

Arrangement referring chart 10	No. of random arrangements with variance	
	Greater	Less
1	176	224
2	147	253
3	310	90
4	281	119

Table VIII again shows that the claim of greater accuracy for balanced arrangements does not hold good for all balanced arrangements. It is possible to have several balanced arrangements and out of them some are more accurate while others are less accurate. In the present instance two cases are in favour and two are against balanced arrangements.

For four treatments in chart 3, balancing has been done in two directions, i.e. along rows and columns. Comparing this arrangement with the 300 random ones, it was found that 199 of them gave greater residual variance and the remaining 101 less. This finding is against the advantages claimed for balanced experiments.

Table IX gives the average treatment error and its variance for the 25 balanced arrangements and the random arrangements considered for the different cases.

Table IX shows that, excepting the case of six treatments, the average treatment errors are more for balanced arrangements. It may be mentioned that the differences are not significant in any of the cases. The variances for the three cases are almost the same. The treatment error and its variance are less for six treatments only. This leads to the conclusion that, on the whole, balanced arrangements are likely to give less accurate results. It may, however, be mentioned that the evidence available is not sufficient to say definitely one way or the other.

TABLE IX

Average treatment error and its variance for random and balanced arrangements

No. of treatments	Average variance		Variance of variance	
	Balanced	Random	Balanced	Random
4	387	314	44,553	53,452
5	188	151	9,442	5,466
6	84	102	1,538	3,819
7	114	104	2,308	3,246

The discussions in the preceding paragraphs show that it is difficult to say in advance which type of arrangement is likely to give more reliable information. During the course of the present investigation we have come across with a number of balanced designs which are likely to give more accurate results than random arrangements. But when laying out an experiment, it is not possible to get at this particular design. Even if the balancing principle is adopted, as has already been pointed out, there are different ways of effecting this balance. In laying out an experiment one of the balanced arrangements will have to be selected at random, as in the case of randomized blocks. The present investigation has not given us conclusive evidence to prefer one design to the other. Under these circumstances the best thing appears to be to have a design in which both the principles are combined, and we get it in Latin squares.

SUMMARY

It has been claimed by Gosset that balanced arrangements are likely to be more accurate than random arrangements. The wheat uniformity trial discussed in a previous paper has been used to investigate the relative merits of random and balanced arrangements. The investigation covers the cases of four, five, six and seven treatments with six, six, eight and six replications, respectively, from different sections of the uniformity trial. It has been found that it is very difficult to say which of them is better. However, there is some tendency for the randomized arrangements to give more accurate results.

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III. DISTRIBUTIONS OF VARIANCES AND RATIO OF VARIANCES

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IT is well known that the ratio of variances test generally used for examining the significance of biological and agricultural data is based on the 'normal' theory. In actual practice the distribution of the parent population may either be not 'normal' or not known. In the case of field experiments we are not in a position to have any correct idea of the distribution of the population. In such cases it is essential to know whether the test usually applied is valid or not. This can be seen by examining the distribution of the ratio of variances (i) between and within groups of samples drawn from the same population, the distribution of which is unknown and (ii) between dummy treatments and residual error for the case of a uniformity trial data, distributing the treatments at random. As regards (i) it has already been shown by Pearson [1931] both from theoretical and experimental points of view that the distribution of the ratio of variances between and within groups of samples from the same population is not very sensitive to change in the population form and that the 'normal' theory tests can be used with greater confidence than others, when dealing with populations whose distribution laws are unknown. Regarding (ii) Eden and Yates carried out some investigations on the validity of Fisher's z -test when applied to an actual example of non-normal data. The data consisted of height measurements of barley selected at random from various nitrogenous fertilizers, and they found the observed distribution agreeing satisfactorily with the theoretical distribution. But they have not clearly mentioned how the 24 permutations of the treatments A, B, C and D have been arranged and in the absence of this information it is not known whether the samples are biased or not.

In the present paper an attempt has been made to get some information regarding the validity of the ratio of variances test as applied to randomized blocks on the basis of the wheat uniformity trial, i.e. on item (ii) mentioned above. Incidentally the distributions of the variances for the dummy treatments and the residual error have also been compared with those of the 'normal' theory.

MATERIAL

Random arrangements for the different cases mentioned in part II have been taken advantage of to examine the agreement or the divergence between the theoretical distributions of the variances and the ratio of variances as compared to what is actually observed under field conditions.

METHODS AND DISCUSSIONS

Variances.—The frequency distributions of the ratio of the variances of the different samples to their mean value have been given in Tables I—IV for the treatment and the residual errors for the four cases discussed in part II. The expected values are calculated by using the distribution la

variances on the basis of the 'normal' theory. It will be seen that this distribution will involve the variance of the population which is unknown. This has been assumed to be equal to the mean variance for the different samples. The error involved in such an assumption can be shown to be very small.

TABLE I
Frequency distribution
(4 treatments \times 6 replications)

For treatment error				For residual error			
Classes	Observed frequency	Calculated frequency	$\frac{(O-C)^2}{C}$	Classes	Observed frequency	Calculated frequency	$\frac{(O-C)^2}{C}$
0—25	6	8.7	0.838	0—170	3	23.7	35.273
50	14	14.1	0.001	180	2	6.3	
75	16	16.5	0.015	190	1	6.9	
100	16	17.1	0.071	200	2	7.8	
125	16	17.4	0.113	210	2	8.7	
150	12	16.8	1.371	220	2	9.0	5.44
175	20	16.5	0.742	230	3	9.6	4.54
200	12	15.6	0.831	240	8	10.2	0.47
225	19	14.7	1.258	250	5	10.8	3.11
250	13	13.8	0.046	260	7	10.8	1.34
275	7	12.9	2.698	270	7	10.8	1.34
300	15	12.0	0.750	280	8	11.1	0.87
325	7	11.1	1.514	290	18	10.8	4.80
350	20	10.2	9.416	300	22	10.8	11.61
375	11	9.6	0.204	310	26	10.5	22.88
400	8	8.7	0.056	320	24	10.2	18.67
425	11	7.8	1.313	330	18	9.9	6.63
450	12	7.2	3.200	340	33	9.6	57.04
475	6	6.6	0.055	350	32	9.3	55.41
500	9	6.0	1.500	360	32	8.7	62.40
525	4	5.4	0.363	370	30	8.1	59.21
550	6	5.1	0.159	380	15	86.4	59.00
575	3	4.5	0.500				
600	3	4.2	0.343				
625	3	3.6	0.100				
650	6	3.3	2.209				
675	2	3.0	0.333				
700	1	3.0	1.333				
725	3	2.4	0.150				
750	5	2.1	4.005				
775	0	2.1	2.100				
800	2	1.8	0.022				
900	4	5.7	1.089				
1000	4						
Upwards	4	6.9					
Total	300	300	38.698	Total	300	300	410.03

TABLE II

Frequency distribution(5 treatments \times 6 replications)

For treatment error				For residual error			
Classes	Observed frequency	Calculated frequency	$\frac{(O-C)^2}{C}$	Classes	Observed frequency	Calculated frequency	$\frac{(O-C)^2}{C}$
0—20	9	12.0	0.750	0—96	4	47.6	45.306
40	24	28.0	0.571	99	3	6.8	
60	21	36.0	6.250	102	2	7.6	
80	41	38.4	0.176	105	3	8.0	3.125
100	42	38.4	0.338	108	3	8.4	3.471
120	44	35.6	1.982	111	3	9.2	4.178
140	36	32.8	0.312	114	4	9.2	2.939
160	24	28.8	0.800	117	3	9.6	4.538
180	32	25.2	1.835	120	10	10.0	0
200	22	21.6	0.007	123	8	10.1	0.437
220	27	18.0	4.500	126	5	10.4	2.804
240	9	15.6	2.792	129	11	10.5	0.024
260	19	12.8	3.003	132	12	10.6	0.185
280	6	10.8	2.133	135	8	10.6	0.638
300	13	8.8	2.005	138	18	10.6	5.166
320	7	6.8	0.006	141	26	10.5	22.881
340	5	6.0	0.167	144	8	10.4	0.554
360	5	4.8	0.008	147	29	10.3	33.950
380	4	4.0	0	150	16	10.1	3.447
400	3	3.2	2.010	153	26	9.9	26.183
420	3	2.4		156	22	9.6	16.017
440	2	2.0		159	39	9.2	96.526
500	2	8.0		162	29	9.2	42.613
				165	34	8.8	72.164
				168	28	8.4	45.733
				171	13	8.4	2.519
				174	20	7.6	20.232
				177	12	7.2	83.959
				180	1	101.2	
Total .	400	400	29.645	Total .	400	400	539.589

TABLE III
Frequency distribution
 (6 treatments \times 8 replications)

For treatment error				For residual error			
Classes	Observed frequency	Calculated frequency	$\frac{(O-C)^2}{C}$	Classes	Observed frequency	Calculated frequency	$\frac{(O-C)^2}{C}$
0—20	9	14.4	2.025	0—80	5	66.7	68.347
25	6	8.8	0.891	82	3	9.8	
30	8	10.4	0.554	84	2	10.7	
35	8	11.6	1.117	86	4	11.2	
40	20	13.2	3.503	88	5	11.7	3.869
45	19	13.7	2.050	90	10	12.2	0.390
50	19	14.5	1.397	92	11	12.5	0.189
55	12	14.8	0.530	94	9	12.9	1.174
60	11	15.0	1.067	96	23	13.1	7.586
65	18	15.1	0.557	98	19	13.1	2.646
70	15	15.0	0	100	22	13.1	5.992
75	16	14.7	0.115	102	24	13.1	9.140
80	13	14.4	0.136	104	32	12.9	28.435
85	14	14.0	0	106	36	12.6	43.314
90	14	13.6	0.012	108	38	12.3	53.698
95	12	12.8	0.050	110	45	12.0	91.274
100	12	12.8	0.050	112	40	11.6	70.078
105	13	11.6	0.169	114	47	11.0	117.302
110	13	11.6	0.169	116	16	10.5	2.841
115	12	10.4	0.246	118	9	117.0	99.692
120	7	10.0	0.900				
125	9	10.0	0.100				
130	7	8.8	0.368				
135	11	8.4	0.805				
140	4	7.6	1.705				
145	8	7.6	0.021				
150	7	6.8	0.006				
155	6	6.4	0.025				
160	10	5.6	3.457				
165	7	5.6	0.350				
170	8	5.2	1.508				
175	6	4.8	0.300				
180	3	4.4	0.445				
185	3	4.0	0.250				
190	3	3.6	0.100				
195	5	3.2	1.013				
200	3	3.2	0.013				
205	4	2.8	0.514				
210	5	2.8	1.729				
215	1	2.4	0.817				
upwards .	19	24.4	1.195				
Total .	400	400	30.259	Total .	400	400	610.630

TABLE IV
Frequency distribution
(7 treatments \times 6 replications)

For treatment error				For residual error			
Classes	Observed frequency	Calculated frequency	$\frac{(O-C)^2}{C}$	Classes	Observed frequency	Calculated frequency	$\frac{(O-C)^2}{C}$
0-10	2 }	1.6 }	0.233	0-75	5 }	48.0 }	45.563
20	5 }	6.8 }		77	2 }	7.6 }	
30	17	14.4	0.469	79	3 }	8.4 }	2.618
40	26	21.2	1.087	81	4 }	8.8 }	
50	13	26.4	6.802	83	5	9.6	2.204
60	29	29.2	0.001	85	4	10.0	3.600
70	32	31.2	0.021	87	1	10.5	8.595
80	26	30.8	0.748	89	3	11.0	5.818
90	25	29.6	0.715	91	10	11.3	0.150
100	35	28.0	1.750	93	7	11.5	1.761
110	21	25.6	0.827	95	15	11.8	0.868
120	32	23.2	3.338	97	17	11.9	2.186
130	28	20.4	2.831	99	19	12.0	4.083
140	14	18.0	0.889	101	15	12.0	0.750
150	20	15.2	1.516	103	28	11.9	21.782
160	19	13.2	2.548	105	30	11.7	28.623
170	12	11.2	0.057	107	24	11.6	13.255
180	8	9.6	0.267	109	32	11.2	38.629
190	9	8.0	0.125	111	29	11.2	28.289
200	3	6.8	2.124	113	24	10.8	16.133
210	1	5.6	3.779	115	32	10.4	44.861
220	4	4.4	0.036	117	29	9.6	39.204
230	5	3.6	0.544	119	16	9.6	4.267
240	4	3.2	0.200	121	24	9.2	23.809
250	3 }	2.4 }	0.613	123	15	8.8	4.368
260	2 }	2.0 }		125	6	8.0 }	86.092
270	1 }	1.6 }		127	1	91.6 }	
Upwards	4 }	6.8 }					
Total	400	400	31.520	Total	400	400	427.508

The values of χ^2 between the observed and the theoretical frequency distribution for the residual and the dummy treatment variances are given in Table V.

Table V shows that the observed distribution of the residual variance is not in accordance with the theoretical distribution. It is interesting to note that the observed distribution of the variance for dummy treatments is not significantly different from that of the theoretical distribution. This might probably be due to the fact that we are dealing with the distribution of variances based on the means.

TABLE V
 χ^2 for the treatment and the residual variances

No. of treatments	Residual variance		Variance for dummy treatments	
	Observed	Theoretical at 5 per cent level	Observed	Theoretical at 5 per cent level
7	427.5	40.1	31.5	41.3
6	610.6	31.4	30.3	> 43.8
5	539.6	42.6	29.6	35.2
4	410.0	33.9	38.7	> 43.8

Ratio of variances.—We have now seen that of the two variances one follows the 'normal' theory, while the other does not. It is now worth while to examine the effect of this deviation on the ratio of variances test. Table VI gives the observed and the theoretical frequencies of the ratio for the four hypothetical experiments discussed before. χ^2 and $P(\chi^2)$ have also been given at the end of the table.

From Table VI it is clear that there is no reason to believe that the ratio of variances test is inapplicable in the case of data the distribution of which is unknown.

This conclusion is in full agreement with those of Pearson, and Eden and Yates. Pearson [1931] after extensive investigations on some non-normal data came to the conclusion that the analysis of variance is applicable over a fairly wide range of non-normality, provided the degree of freedom for the residual error is not small. Eden and Yates [1933] also found the distribution of z for 1000 random samples agreeing satisfactorily with the theoretical distribution.

SUMMARY

The distributions of variances for (i) the treatments, (ii) the residual error and (iii) their ratio, have been investigated for experiments involving four, five, six and seven treatments, distributing dummy treatments at random on data computed from a uniformity trial. The treatment variances are distributed in accordance with the 'normal' theory, while it is not so in the case of the residual error. As regards the ratio, the observed distributions are fairly in agreement with the theoretical distributions on the basis of the 'normal' theory.

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RESEARCH NOTE

A MOSAIC DISEASE OF COWPEA

BY

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(With Plates VII and VIII and two text-figures)

URING the study of the effect of mixed cropping on the incidence of root-rot disease of cotton at Lyallpur an experiment was laid out in which Punjab cowpea type 1 (*Vigna Catiang*) had been inter-cropped in between the rows of *desi* cotton variety Mollisoni 39. In addition, a border cowpea 2 ft. in width was sown all round the mixed plots. Both cotton and cowpea were sown on 20 May 1940. The affected cowpea plants exhibited three groups of symptom pictures. The first symptoms of the disease described in this note appeared about five to six weeks after sowing. Cowpea plants at this stage had almost covered the entire cotton crop as well as the surface of the soil in mixed plots.

GROUP I: The most obvious symptom is the general stunting of the affected cowpea plant. The symptoms are more marked in the upper portion of the plant. A prominent feature is the appearance of thick wrinkled veins. The veins of the affected leaves are usually translucent when seen in bright light. After sometime mottling of leaves appears. This consists of small irregular light green areas which in parts are almost devoid of chlorophyll. Earlier on these turn yellow and the leaf-surface at this stage is a combination of yellow and green patches. The dark green areas are sometimes raised and look like blisters. The yellow patches are more marked on the upper surface of the affected leaf. Mottling and deformity of the leaf is usually accompanied by waving of the margins.

GROUP II: In some plants universal yellowing of the leaves is more prominent and the leaves are neither abnormally thick nor highly distorted.

GROUP III: There are other plants, the leaves of which show yellow green patches. The mottling is very conspicuous. The diseased leaves eventually develop pale to brilliant yellow areas which later turn brownish in parts. The veins, including the mid-rib, turn reddish and look like dark streaks. The most characteristic feature is the presence of dark brown reddish spots about 1—2 mm. across on the upper surface of the affected leaves.

The common feature is the general stunting of the diseased plants and affected leaves in due course drop off. Plate VII, figs. 1 and 2 and Plate VIII show symptoms exhibited by three groups of plants.

It may be mentioned that the naturally infected plants were seen both in the Botanical Experimental area and the Students Farm at Lyallpur where cowpea (Punjab type 3) alone had been sown. In the mixed cropping experiments the cotton plants appeared to be quite healthy and normal. About 15 per cent of the cowpea plants were affected.

Histology

Transverse sections of the young wrinkled diseased leaves taken from the upper portion of the plant showing stunting and symptom picture of the first group and also leaves of a healthy plant were cut and examined microscopically. The affected leaf is much thicker than the normal leaf. The margin of the affected leaf is irregular and wavy and the cuticle is fused with the epidermal layer at various places; the epidermal cells are not defined but the cuticle and the epidermis is regular and marked in the healthy leaf which has a regular outline. In the affected leaf the normal palisade cells are small in number and the palisade tissue is neither continuous nor regular whereas in the case of unaffected leaf the palisade tissue is regular. The palisade cells of the affected leaf have very few chloroplasts, whereas those of healthy leaves are full of them. The spongy parenchyma in the affected leaf is adversely affected. The sclerenchyma cells of the diseased leaf are thicker and larger in size than those of healthy leaves. The vascular bundles in the affected leaves are scattered and are not arranged regularly. The xylem vessels tend to be thicker and larger than in the healthy normal leaf. In the affected leaf a large number of elongated and irregular cells develop which appear to have partly taken the place of spongy and palisade tissues. Figs. 1 and 2 show transverse sections through a diseased and a healthy leaflet respectively.

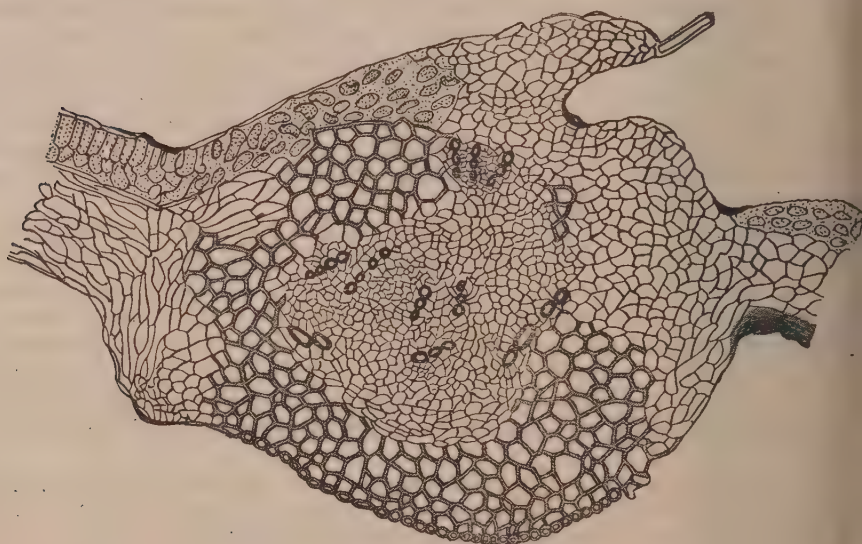


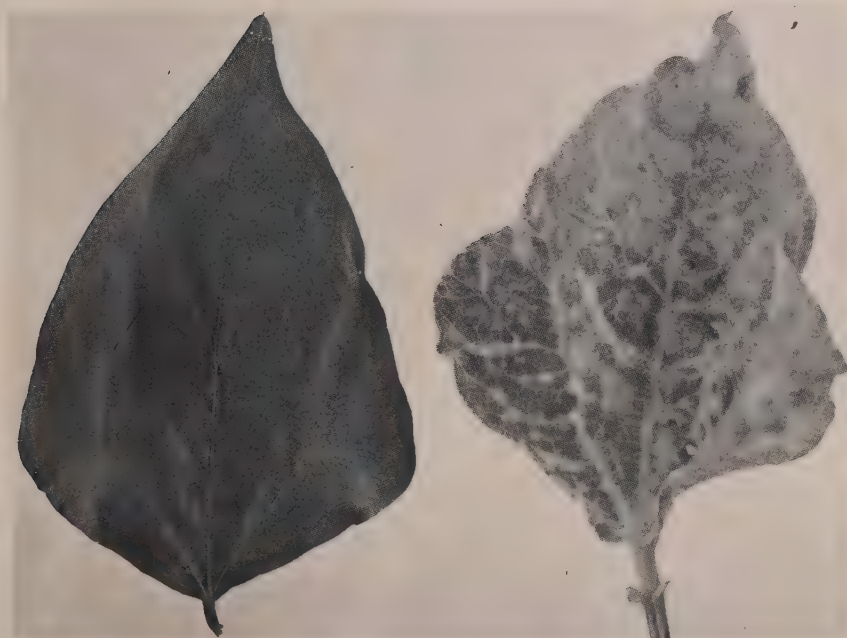
FIG. 1. Transverse section of a malformed cowpea leaflet ($\times 50$)



Healthy

Diseased

FIG. 1. Leaves of group I plants



Healthy

Diseased

FIG. 2. Leaves of group II plants



Diseased

Healthy

Leaves of group III plants

ctivity

Isolations made from the diseased material did not reveal any organism. Microscopic examination also did not reveal the presence of fungal hyphae.

The juice of the plants was extracted in a sterilized mortar and pestle and mortar, strained through fine muslin cloth and centrifuged to remove the

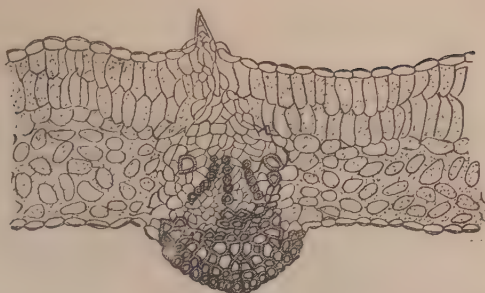


FIG. 2. Transverse section of a healthy leaflet ($\times 50$)

debris. Inoculations were made by smearing the leaves with the juice by means of a spatula on which cloth had been wrapped and pricking the diseased leaves. Juice was also introduced by means of a syringe, but as the first method proved quite handy and successful it was continued. Young pea plants raised in sterilized soil inside large glass-walled chambers were inoculated with the extracts of the washed diseased plant leaves. Plants were inoculated with the juice of healthy plants.

The juice from leaves of plants showing all the three types of symptoms described above was used separately for inoculation purposes, but it was observed that in all cases yellow mottling appeared on the leaves of inoculated plants within five days, whereas the controls remained healthy. In these preliminary inoculation tests the distortion of the leaves was not observed and have been reproduced.

Holmes [1939] mentions a mosaic disease of cowpea induced by artificial inoculation with cucumber-mosaic virus, cowpea-mottling strain, but does not mention the occurrence of the disease in nature. It has not been determined whether the disease referred to in the note is the same. Smith [1924] however, mentioned the occurrence in Louisiana, Arkansas and Indiana of cowpea mosaic causing mottling and crinkling of the leaves.

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REVIEWS

Annual Review of Biochemical and Allied Research in India, Vol. XI, 1940

Society of Biological Chemists, India, Bangalore : Price Rs. 3 or 6d.

THE review covers a wide field and consists of 16 sections the subject-matter of which has an important bearing on agricultural science, such as enzymes, vitamins, animal nutrition, adulteration of foods, phytopathology, soil fertilizers and manures, biochemical and allied industries, etc. Only a brief mention of a few of the items is made in this short review.

The contributions made in the field of enzymes during the year relate to the nature of carboxylase, zymohehexase, liver aldehyde oxidase, cytochrome oxidase and other oxidizing enzymes. The work of Indian workers from the Biochemical Laboratories, Cambridge, on biological oxidations, also deserves notice.

The work of the Bengal Nutrition Committee and the occurrence of widespread famine in the south-eastern districts of the Punjab raise the question of Vitamin-A deficiency. In the famine areas scurvy and night blindness have been widespread. While scurvy was largely controlled by the use of *amla* powder, no such cheap and potent source of vitamin-A was available for mass distribution. War stopped the import of cod-liver oil. Scientists therefore began to look to the life in the numerous rivers, bay canals and tanks of India for any available supply of vitamin A.

In 1940, there was greater interest in putting down the adulteration of food and drugs. The enactment of the Drugs Act and the formation of the Central Committee for Food Standards were notable events. Equally encouraging was the output of scientific work in this field.

The year marks an increasing interest in researches on applied plant physiology, such as in vernalization, water relation, the influence of mineral elements, etc. Useful contributions were made on germination and viability of seeds, respiration in light, photo-periodism and radiation effects on the growth of plants.

In the field of entomology, several studies were made dealing with insect pests of cotton and sugarcane. Further, *Schistocerca gregaria* Forsk. has been found to be the locust *par excellence* of India not only by the frequency of its visitations, but also by the extent and severity of its attacks. The locust problem is now recognized to be an international one and in setting out India's part in investigations on this pest, considerable advance has been made in the study of the bionomics of the desert locust, the nature of its habits and the conditions under which new outbreaks may occur.

The second world war has brought in its train great slackening of all scientific research except that connected with the war effort. This has been due to the difficulties in obtaining requisite supplies of suitable chemicals and apparatus. In the domain of the chemistry of plant products, the paucity of research by Indian chemists is particularly noticeable during the year.

ever, important investigations have been carried out in this line on essential oils, fixed oils and waxes, lactones and glucosides comprising bitter principles of plants, plant-colouring matters and alkaloids.

Under biochemical and allied industries, interest continues to be centred on the utilization of molasses for the preparation of chemicals. The spade work in connection with the manufacture and use of power alcohol may now be considered to be complete with the enforcement of the U. P. Power Alcohol Act of 1940, making it compulsory for every petrol distributor within certain limits to add 20 volumes of power alcohol to every 80 volumes of petrol, before supplying the latter to the public. The industry has, however, received a temporary setback, as it is not possible to import the necessary plant.

The interest in the manifold problems presented by the soils has continued to manifest in the impressive volume of work in the year.

A comparative study of the different soils of India and profiles for their classification into broad groups in relation to the world scheme of classification as to the cultural and fertilizer practices was in progress. In order to get a general idea of the evolutionary status of the Indian soils under varied biological and climatic influences, three soil maps have been prepared there, based on (1) agricultural and colour nomenclature, (2) the relative nitrifying power of surface soils, and (3) climatic differences.

Molasses, a waste product of the sugar industry, has found useful applications as manure, and so also filter-press cake, and compost made up of pressed cane-trash and bagasse.

Fertilizer trials were continued during the year. In Bihar an application of 60 lb. of N and 60 lb. of P_2O_5 to sugarcane gave a net profit of Rs. 35-40 per acre, in the Central Provinces the highest net profit per acre was with 20 lb. of P_2O_5 and amounted to Rs. 3-12 only per acre; while in Orissa doses of nitrogen from 20 to 40 lb. gave increased yields which, however, did not cover the cost of manure. The application of phosphatic manures to Assam gave profitable response in crop yields.

Dry cultivation offers the largest scope for increasing the wealth of the country, especially in Mysore where 80 per cent of land is under dry cultivation.

An Agricultural Testament by Sir Albert Howard published during the year is a useful contribution to the careful study of Indian agricultural problems. The whole thesis of the publication is to show that for true agricultural success organic manure is essential, since it produces humus, and humus is necessary for mycorrhizal symbiosis between the plant roots and the soil. An extensive experience has apparently shown to be fundamental. Plants grown under proper agricultural conditions, with ample aeration in presence of humus, are shown to be disease-resistant, and animals including human beings fed on such vegetables are also resistant to disease (B. V. N.).

NOTES

BOMBAY AGRICULTURAL PESTS AND DISEASES ACT 1941

THE *Bombay Government Gazette* of the 12th September 1941 publishes an Act to provide for the prevention of the introduction, spread or reappearance of insect pests, plant diseases and noxious weeds injurious to crops, plants or trees in the province of Bombay, to be known as the 'Bombay Agricultural Pests and Diseases Act, 1941'.

Four distinct forms of action under the Act for control of pests, diseases or weeds are envisaged. The provincial Government may, by notification in the official Gazette :—

- (1) declare that such pest, disease or weed is an insect pest, plant disease or noxious weed
- (2) specify the local area within which and the period during which such declaration shall remain in force
- (3) prohibit or restrict the removal of any plant or tree from one place to another, and
- (4) direct the carrying out of such preventive or remedial measures including the destruction of any pest, disease or noxious weed on any crops, plants or trees, as the provincial Government may deem necessary, in order to eradicate such pest, disease or weed or to prevent its introduction, spread or reappearance

The Government will appoint Inspectors to enter upon any land or premises within a notified area, to ascertain the presence of insect pests, plant diseases or noxious weeds and to see that the measures advocated have been carried out. If the measures have not been taken, he is empowered to issue an order giving a time limit for their completion, against which order an appeal may be preferred with the Collector. In the event of continued failure, the Inspector himself may carry out the work, the cost being recovered as an arrear of land revenue. Compensation for trees or plants destroyed under a general or particular order will be granted. The amount of compensation shall be as follows :—

- (1) If a tree is infected with an insect pest or a plant disease, a sum not exceeding half the value of the tree.
- (2) If plants are grown so close together that they cannot be treated individually and healthy plants have also to be destroyed, a sum not exceeding three-fifths of the value.
- (3) If plants or trees are destroyed which though not infected at the time with an insect pest or a plant disease are, in the opinion of the Inspector, liable to such infection, a sum equal to the value.

Cotton plants are excluded from compensation, as also plants and trees which in the opinion of the Inspector contracted infection due to negligence of the occupier in carrying out preventive or remedial measures mentioned in a notification.

Persons removing plants or trees in contravention of a notification, or failing to comply with a notice, or in any other way committing a breach of the provisions of the Act are liable to punishment with a fine which may extend up to Rs. 25.

The Act itself does not include the mention of any particular insect pest, disease, or weed, but a statement appended to the notification mentions a number of pests and diseases requiring attention, namely mildew, aphids, borers, cotton bollworm, grasshopper, coconut-tree pests, and *koleroga* on the use of betel-nut palms. It is mentioned that owing to the failure of a few owners or occupiers to cooperate, it has been impossible to eradicate the pests and diseases effectively, and it is in order to compel simultaneous action that the Act has been passed.

This Act appears to be modelled on the 'Madras Agricultural Pests and Diseases Act, 1919', which has proved of considerable help to the Madras Department of Agriculture in enforcing pest control measures in that province.

MAYNARD-GANGA RAM PRIZE

APPLICATIONS are invited for the award of the Maynard-Ganga Ram Prize of Rs. 3,000 for a discovery or an invention or a new practical method which will tend to increase agricultural production in the Punjab on a permanent basis. The prize is open to all, irrespective of caste, creed or nationality, and Government servants are also eligible for it. Essays and theses are not accepted. The prize will be awarded for something practically achieved as a result of work done after the prize was founded in 1925. In their applications competitors must give a clear account of the history of their invention or discovery and must produce clear evidence that it is the result of their own work. In the case of an improved crop details of parentage, evolution of history, and a botanical description are necessary.

The Managing Committee reserves to itself the right of withholding or postponing the prize if no satisfactory achievement is reported to it, or to reduce the amount of the prize or to divide it if the quality of the entries justifies such action.

Entries should reach the Director of Agriculture, Punjab, Lahore, not later than 31 December 1942.

THE Imperial Agricultural Bureaux have just issued the 10-year Subject and Author Index to *Horticultural Abstracts* 1931-40. Price about 25s. (free issue.)

All orders should be sent direct to The Imperial Agricultural Bureaux, Central Sales Branch, Agricultural Research Building, Penglais, Aberystwyth, Wales.

PLANT QUARANTINE NOTIFICATIONS

DIA

Form of special permit authorizing importation of insects

prescribed by the Central Government under para. 2(a) of the Notification*
No. F.-193/40-A, dated 3 February 1941]

Name, designation and full address of the importer

Name of the insect species to be imported

Stage or states of the insect to be imported

Country from which importation is sought

Whether importation is intended by sea, land or air

Whether in its original home it is a weed pest, a parasite or a predator.

(i) Name (names) of the weed (weeds) on which it is a pest in the country of origin

(ii) Name (names) of the pest (pests) on which it is a parasite or predator in the country of origin

Name, designation and address of the exporter

Quantity indented for

Purpose of importation

I authorize the importation. This permit will be valid up to

(Signature and designation of the
certifying authority)

te.

[N.B.—It is expected that the permit will be obtained in advance of sending the letter so that the imported material may not remain indefinitely in the warehouse for want of suitable permit.]

Notification No. F. 193/40-A. (c), dated 12 August 1941 of the Government of India in the Department of Education, Health and Lands

exercise of the powers conferred by sub-section (1) of section 3 of the Destructive Insects and Pests Act, 1914 (II of 1914), the Central Government pleased to direct that the following amendment shall be made in the Order

*Published in this Journal, Vol. 11, Part II, page 322

published with the notification of the Government of India in the Department of Education, Health and Lands, No. F.-193/40-A., dated the 3rd February 1941, namely :—

In clause (b) of paragraph 3 of the said Order, after the word ' Orissa ' the words ' Jammu and Kashmir ' shall be inserted.

Notification No. F. 15-11/41-A., dated 1 September 1941 of the Government of India in the Department of Education, Health and Lands

IN exercise of the powers conferred by sub-section (1) of section 3 of the Destructive Insects and Pests Act, 1914 (II of 1914), the Central Government is pleased to direct that the following further amendment shall be made in the Order published with the notification of the Government of India in the Department of Education, Health and Lands, No. F. 320-35-A. dated the 20th July 1936, namely :—

In sub-paragraph (2) of paragraph 9 of the said Order for the words and brackets ' (*Ceratostomela paradoxa* or *Thielaviopsis paradoxa*) ' the words and brackets ' *Ceratostomella paradoxa* (*Thielaviopsis paradoxa*) ' shall be substituted.

FOREIGN COUNTRIES

Notice No. 2 of 1941 regarding plant quarantine regulations and import restrictions received in the Imperial Council of Agricultural Research

THE following plant quarantine regulations and import restrictions have been received in the Imperial Council of Agricultural Research. Those interested are advised to apply to the Secretary, Imperial Council of Agricultural Research, New Delhi, for loan.

1. Quarantine and other official announcements

- (i) Service and Regulatory Announcements October-December 1940
- (ii) Fruit and Vegetable Quarantine of Puerto Rico
- (iii) Japanese Betel Quarantine

2. Summaries of plant quarantine import restrictions

- (i) Plant Quarantine import restrictions of the Dominion of Canada
- (ii) Plant Quarantine and Import Restrictions of the Free City of Danzig previous measures abrogated
- (iii) Foot-and-mouth disease in Norway

3. Other announcements

- (i) Government of Burma, Department of Agriculture and Forests Notification No. 141, dated the 2nd June 1941
- (ii) Government of Burma, Department of Agriculture and Forests Notification No. 182, dated the 25th June 1941 regarding import of live insects into Burma

ERRATA

THE INDIAN JOURNAL OF AGRICULTURAL SCIENCE, VOL. IX, PART IV

page 605—

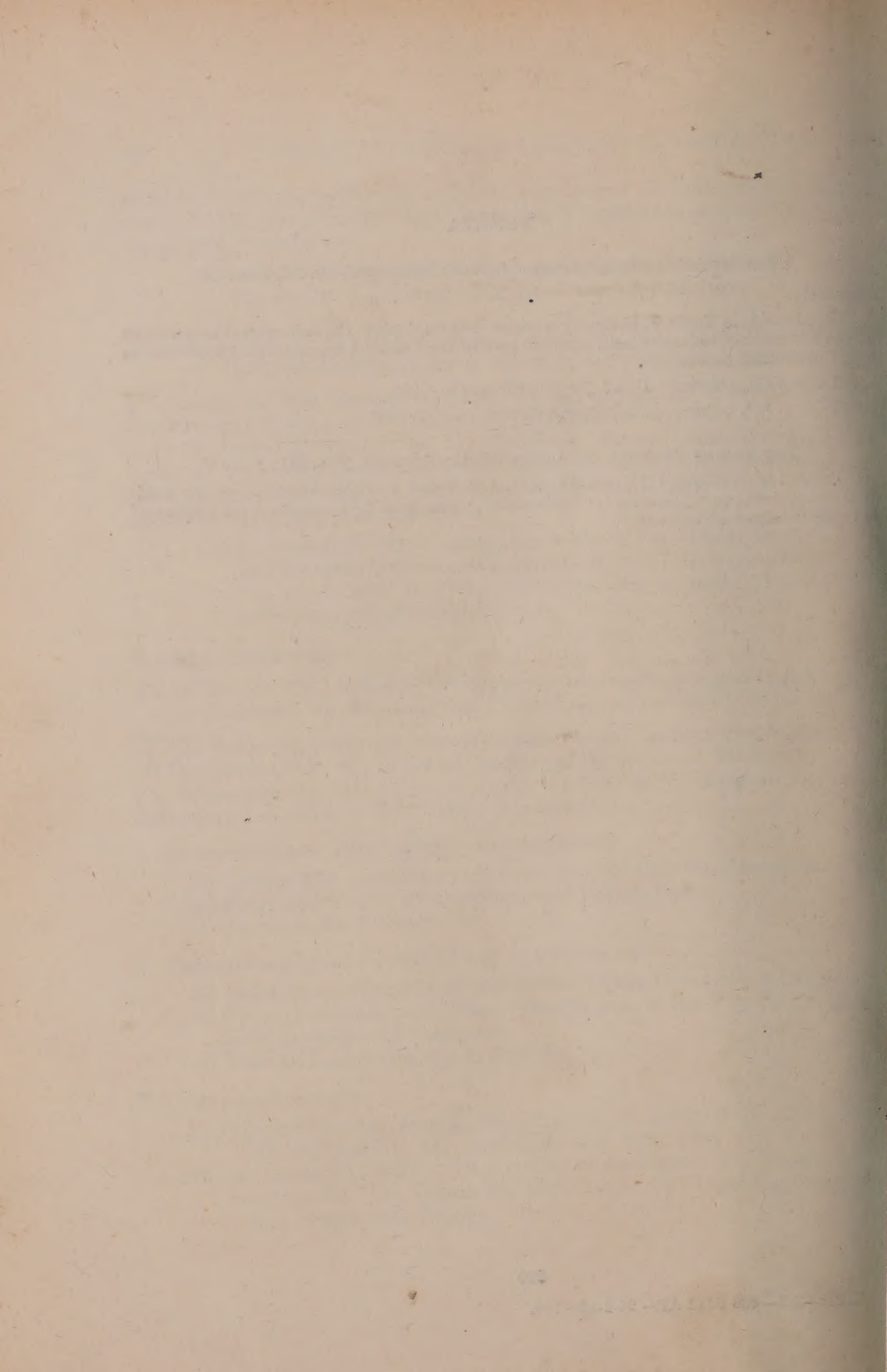
Total solids in Table VIII signify residue left on drying the soil extract to constant weight and are not to be confused with the sum of the different water-soluble constituents each determined separately.

Table VIII, column 2, line 7, for '0·010' read '0·102'

Table VIII, column 9, line 5, for '0·095' read '0·038'

THE INDIAN JOURNAL OF AGRICULTURAL SCIENCE, VOL. XI, PART V

Pages 710-11 (Table III), columns 3, 4, 5, 6, 7 and 8 of the lower half of the table refer respectively to '*Hirsutum*', '*Herbaceum*', '*Arboreum*', '*Cernuum*', 'All cottons' and 'By analysis of variance'.



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English Book Depot, Taj Road.
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ELHI—
Imperial Book Depot and Press, Near Jama Masjid (Machhliwalan).
Income-tax Law Publishing House, Chandni Chowk.*
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Curator, Govt. Book Depot, Burma.

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